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(Recognosce notum, ignotum inspice)

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THE ECOLOGY OF SOME LOWER FUNGI AT ESTHWAITE WATER

By L. G. WILLOUGHBY

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(With Plates 22 and 23 and 17 Text-figures)

At Esthwaite Water (English Lake District) the distribution of saprophytic Chytridiales, both above and below the lake margin, was studied. Certain species proved to be essentially terrestrial, others essentially aquatic. Species of both categories were obtained from the lake margin, and there was strong evidence that this situation was an exceptionally favourable one for the growth of saprophytic chytrids.

In recent collections of saprophytic Chytridiales in the English Lake District and elsewhere certain species, such as *Cladochytrium replicatum* Karl., were recovered from submerged lake muds but not from soil. On the other hand, certain typical soil chytrids, such as *Rhizophlyctis rosea* (de Bary & Woron.) Fisch. were never obtained from aquatic situations. These observations suggested that the flora of saprophytic chytrids in these two environments might be quite distinct and different, and margins of bodies of fresh water might be worthy of detailed examination.

With these ideas in mind, therefore, the present investigation was made at a single site on Esthwaite, a small lake in the Lake District. An attempt was made to determine the flora of saprophytic chytrids in soil or mud at three levels on an approximate line transect across the lake margin. The levels investigated were: (i) the field zone, sampled just above the highest recorded lake level; (ii) the marginal zone, periodically inundated when the lake level was high; and (iii) the permanently submerged *Littorella* zone at approximately 2 m. depth. Since the general aim was to enumerate the species with a high constancy of occurrence (see Sewell, 1959) in these three environments, a programme of repeated sampling was started in February 1958. Collections of soil and mud were made in every month, with the prospect of detecting possible seasonal periodicity.

Soil collections from the field zone, used for cow pasture in the summer, were made from the surface 5 cm. The field had a good cover of grasses: *Anthoxanthum odoratum* L., *Arrhenatherum elatius* Beauv., *Dactylis glomerata* L., *Festuca rubra* L., *Holcus lanatus* L., and *Poa trivialis* L. were identified. Marginal collections were made from the thin, highly organic, mud layer overlying gravel in this situation, and colonized by plants of *Phalaris arundinacea* L. Collections from permanently submerged mud were made with a Petersen grab from a point approximately 7 m. from the shore, and just off a reed bed of *Phragmites communis* Trin. Only the top 5 cm. of mud was retained and used, the whole grab collection being rejected if plants of *Littorella lacustris* L. were not obtained. These plants were removed by

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hand before returning to the laboratory. The method of recovery of the fungi from the collections was essentially that used by previous investigators, and consisted in baiting with suitable complex organic substrata in the laboratory. Each soil or mud collection (approx. 150 g.) was mixed thoroughly and portions (approx. 6 g.) placed in a number of separate Petri dishes. Just enough sterile charcoal water (Willoughby, 1956) was added to enable the baits to float on the surface meniscus. The ratio of the amount of soil or mud used to water added was thus deliberately kept as high as possible, with the aim of ensuring that the final composition of the liquid in the dishes depended on the nature of the collected sample rather than on the charcoal water. Determinations of pH indicated that as far as this factor was concerned the aim was achieved. The baits used (Karling, 1945, 1946; Willoughby, 1956, 1958) were: (i) cellulose materials (waterproof cellophane, grass leaves, epidermal strippings from onion bulbs), (ii) chitin materials (purified shrimp exo-skeleton and termite wings), and (iii) keratin material (snake skin). These baits are designated below as *c*, *g*, *o*, *s*, *t* and *n*, respectively. For each of the three zones, two Petri dishes of each bait were prepared. Since it is common for individual chytrid species to be restricted to one type of bait, the pairs of dishes each tended to have different chytrid populations. After an incubation period of approx. 14 days at room temperature the chytrids present were recorded by drawings. In the intervals between examinations, all dishes were stored in a cold room at 5°. It was particularly important to deal with marginal collections in this way, since the rate of decomposition in them was so high that it was not unusual for the baits to disappear completely with longer incubation periods.

The fungi obtained are listed below and are described and illustrated unless agreement with the account cited was so exact that a description of the Esthwaite material would make no further contribution to knowledge. Although this investigation was primarily concerned with Chytridiales, *Blastocladiella britannica* Horenstein & Cantino (Blastocladiales) was also studied, since its monocentric organization is essentially chytrid-like. In the list, the bait or baits on which the particular species was found are recorded immediately after the name; * denotes a new British record.

Species recorded

ASTEROPHLYCTIS SARCOPTOIDES Petersen; *s*, *t*; see Antikajian (1949). Both direct and indirect (Text-fig. 8*a*, *b*) development from the zoospore cyst was observed. Isolated in pure culture on YpSs agar.

BLASTOCLADIELLA BRITANNICA Horenstein & Cantino (1961); *g*, *n*, *t*; see also Willoughby (1959*a*).

**CATENOCHYTRIDIUM CAROLINIANUM* Berdan (1939, 1941); *g*, *o*.

**CHYTRIOMYCES APPENDICULATUS* Karling (1947); *s*, *t*. Text-fig. 8*h*–*j*.

C. AUREUS Karling and *C. HYALINUS* Karling (1945); *s*, *t*. A deep zone of clear cytoplasm below the operculum was frequently observed, especially in thalli referred to *C. hyalinus* (Text-fig. 8*c*). Separate records were kept of thalli with hyaline (*C. hyalinus*) and with golden-red (*C. aureus*) zoospore globules (Text-fig. 8*e*, *f*), but the fact that both occurred together so

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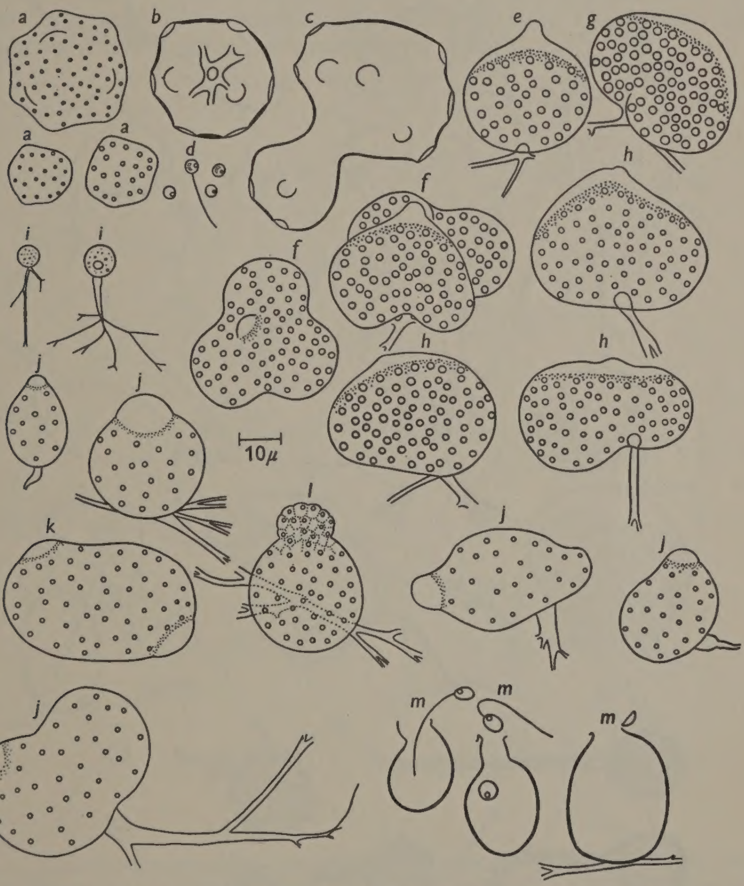
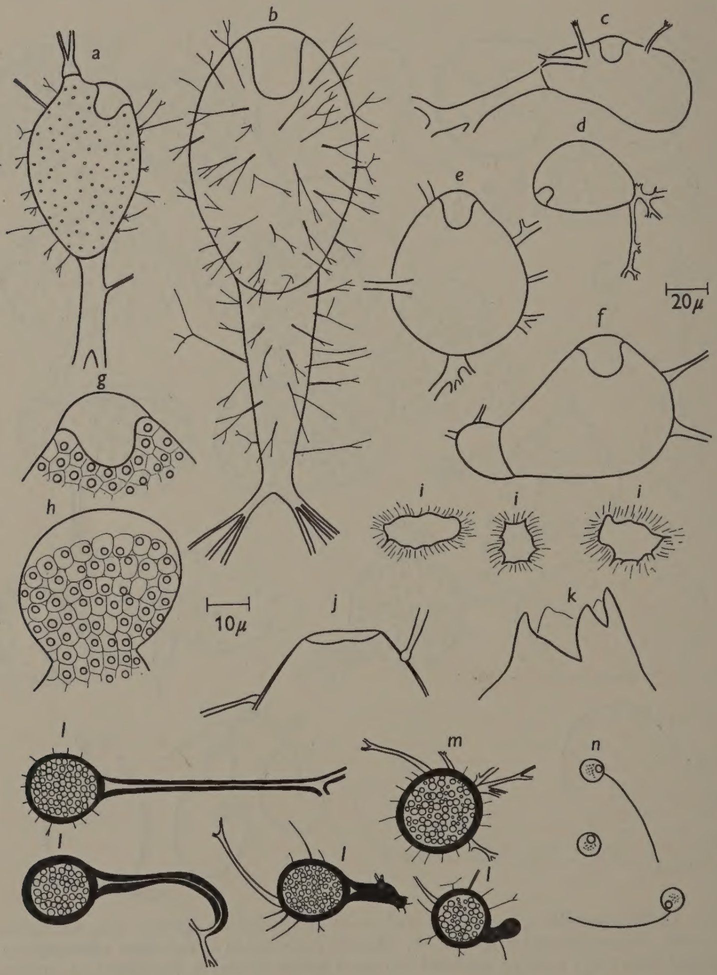
frequently, and thalli with very faint pigmentation were not uncommon, suggested that a single species only might be involved. Thalli with both hyaline and pigmented globules (Willoughby, 1959*b*) were not found. In an attempt to clarify the position, a pure single spore isolate of *C. hyalinus* was studied intensively, but visible pigmentation could not be induced. This isolate produced both nodulated (Text-fig. 8*d*) and non-nodulated sporangia, a result which is relevant to Karling's (1947) discussion of *C. nodulatus* Haskins (1946).



Text-fig. 1. Cellulosophilic species from soil. *a–d*, *Rhizophydium elyensis*; *e–h*, *Rhizidium richmondense*; *i–m*, *Phlyctochytrium irregulare*. *a*, Mature sporangia; *b*, dehisced sporangium showing rhizoidal insertion; *c*, large atypical dehisced sporangium; *d*, zoospores; *e*, typical mature sporangium; *f*, deeply lobed sporangia; *g*, sporangium with the zoospore cyst outline obliterated by growth; *h*, sporangia with cyst outlines partly obliterated by growth; *i*, young thalli; *j*, mature sporangia; *k*, mature sporangium with two dehiscence papillae; *l*, emergence of zoospores; *m*, dehisced sporangia showing opercula or fragments of these at the exit pore rim. All to the same scale.

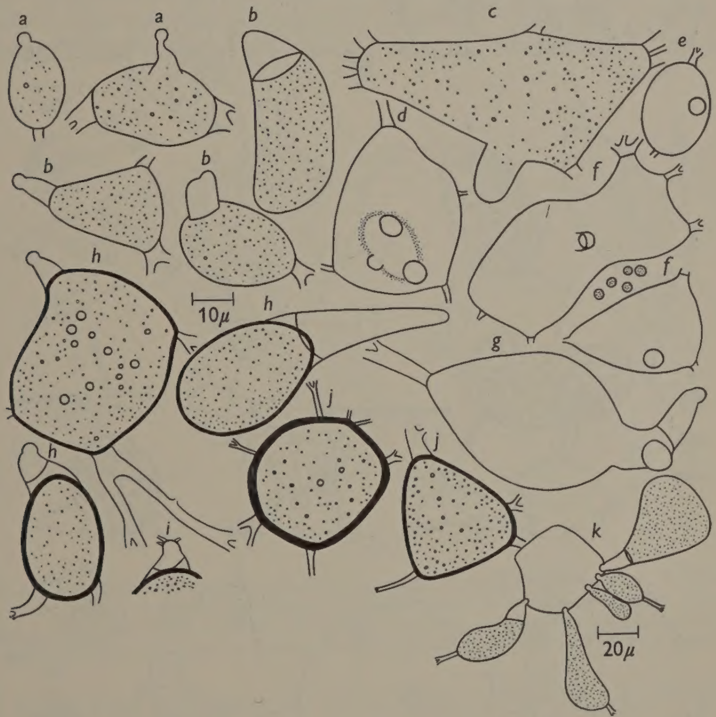
Cladochytrium replicatum Karling (1931); *g*, *o*. Sporangia were 11–17·5 μ diam. (See discussion of *Nephrochytrium aurantium* below.) A study of the nutrition and fruiting behaviour of this species in pure culture has been made and will be reported later.

**DIPLOPHLYCTIS AMAZONENSE* (Karling) Sparrow (1960); *o*. Sporangia globose, 11–75 μ diam., or ovoid 20–53 × 12–35 μ, with an apophysis



Text-fig. 2. Chitinophilic species from soil. *Rhizophlyctis ingoldii*. *a*, Mature sporangium; *b*, same with surface ornamentation of branched hairs; *c–f*, outlines showing range of form, the cell cut off at the left of *f* is devoid of contents; *g*, *h*, emergence of zoospores; *i*, exit pores of dehisced sporangia seen from above; *j*, smooth, and *k*, irregular exit pore rim; *l*, resting spores with one rhizoidal axis grossly thickened; *m*, resting spore; *n*, zoospores. The limit of the zone of clear cytoplasm below the dehiscence papilla is indicated in *a–g*. Scale to the right of *d* for *a*, *c–f*, and *i* only.

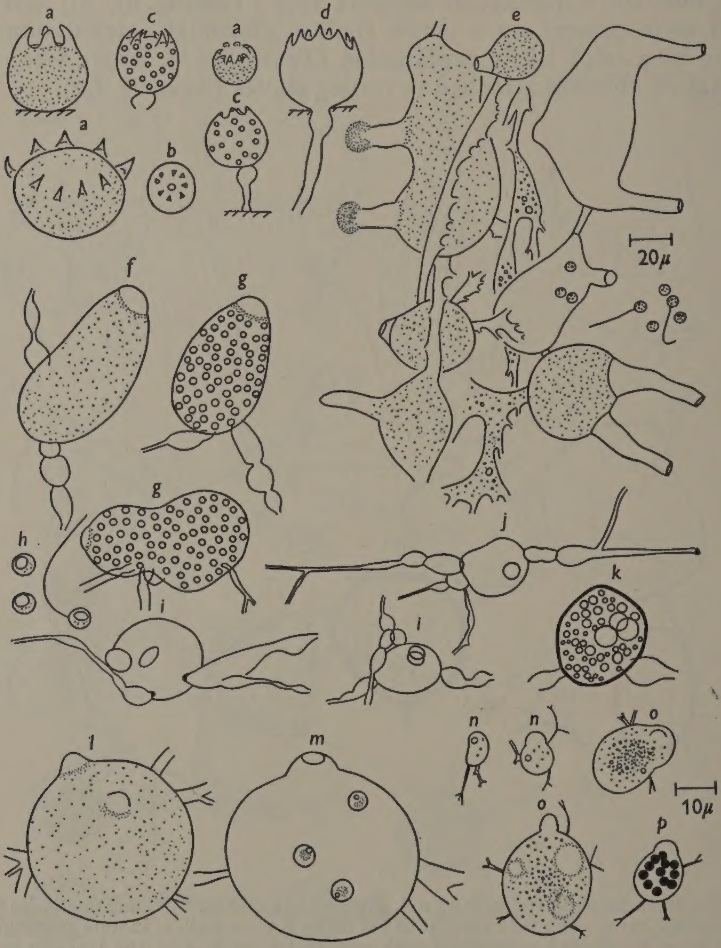
9–12 μ diam. (Text-fig. 6*h–l*). Exit tubes (1 or 2) were 6–150 μ long and 3–35 μ wide with an endo-operculum situated up to 10 μ below the tip. Zoospores 4·5–5 μ diam., with a lateral hyaline globule 2–2·5 μ diam., nuclear cap, and posterior flagellum 27 μ long (Text-fig. 6*k*). Resting spores spherical 15–20 μ diam., with a wall 1 μ thick, spines 2–2·5 μ tall, and an apophysis 5 μ diam. (Text-fig. 6*m*, *n*). Early developmental stages were not observed. A swelling on the rhizoidal system of some sporangia might indicate a persistent zoospore cyst (Text-fig. 6*j*, *k*); but where several were present on a single thallus, rhizoidal catenulation would seem to be a more likely explanation (Text-fig. 6*h*). There were similar swellings on rhizoids attached to resting spores (Text-fig. 6*n*).



Text-fig. 3. Keratinophilic species from soil. *Phlyctorhiza variabilis*. *a*, Young thalli with persistent zoospore cyst; *b*, same with germ tube cut off by a cross wall; *c*, mature sporangium with exit tube; *d*, dehisced sporangium with persistent zoospore cyst and two exit pores; *e*, *f*, dehisced sporangia with no persistent cyst; *g*, dehisced sporangium with persistent cyst and germ tube cut off by a cross wall; *h*, *i*, resting spores with persistent cyst and germ tube; *j*, resting spores with no persistent cyst; *k*, young thalli developing in situ in a dehisced sporangium. Zoospores drawn at *f*. All except *k* to the same scale.

**ENDOCHYTRIUM DIGITATUM* Karling (1938); *g*, *o*. Sporangia globose, 35–45 μ diam., obpyriform 30–55 × 23–35 μ, reniform 30 × 55 μ, or deeply tri-lobed 40–55 μ across, with 1–3 prominent exit tubes (Text-fig. 7*a*–*g*). Exit tubes 12–120 μ long and 6–12 μ wide. Zoospore globule hyaline, 3 μ diam. Operculum and exit pore 5 μ diam. (Text-fig. 7*f*, *g*). Resting spores spherical, 30–35 μ diam., or ovoid 22–35 × 17–25 μ, with a single hyaline globule 12·5–22 μ diam., and a wall 0·5 μ thick (Text-fig. 7*h*, *i*).

Early stages in growth were not observed, and it was therefore not possible to confirm the striking digitations at the base of the developing sporangium reported by Karling. However, certain irregular mature and dehisced sporangia (Text-fig. 7*c*, *e*) suggested that these might well have been present. One irregular exit tube apex (Text-fig. 7*g*) was very similar to Karling's figs. 29 and 34.

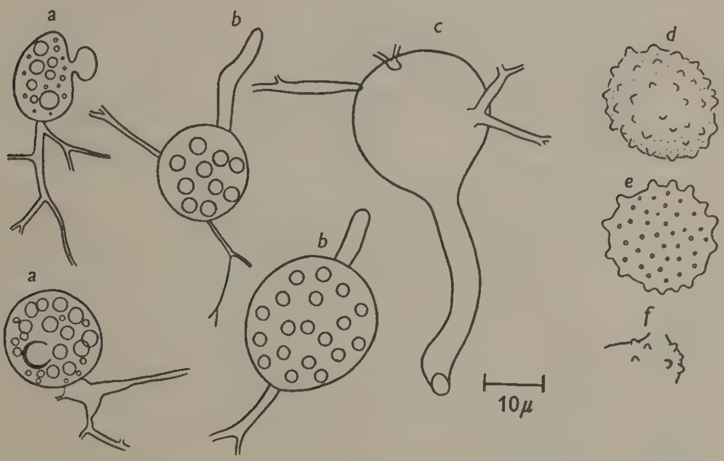


Text-fig. 4. Cellulosophilic species from the lake margin. *a–d*, *Phlyctochytrium planicorne*; *e*, *Rhizophlyctis rosea* (polycentric); *f–k*, *Karlingiomyces marilandicus*; *l*, *m*, *Rhizophlyctis* sp. 2; *n–p*, *Entophlyctis* sp. 2. *a*, Young sporangia; *b*, one from above; *c*, mature sporangia with apophyses; *d*, one in outline; *e*, composite habit drawing; *f*, young sporangium; *g*, mature sporangia; *h*, zoospores; *i*, dehisced sporangia with detached opercula; *j*, a dehisced sporangium; *k*, resting spore; *l*, young sporangium; *m*, dehisced sporangium with zoospores; *n*, young thalli with persistent zoospore cyst; *o*, young thalli with persistent cyst and central pigmentation; *p*, a mature sporangium. Scale of *e*, *f*, *g*, *i–k* to right of *e*; scale of all others to right of *o*.

Entophlyctis sp. 1; *o*. Sporangia globose 15–23 μ diam., with a prominent exit tube and 1–3 separate rhizoidal axes. Exit tubes 10–30 μ long × 3·5–5 μ wide (Text-fig. 5*b*, *c*). Zoospore globules hyaline, 2–2·5 μ diam. A persistent zoospore cyst 4–5·5 μ across occurred on young thalli (Text-fig. 5*a*).

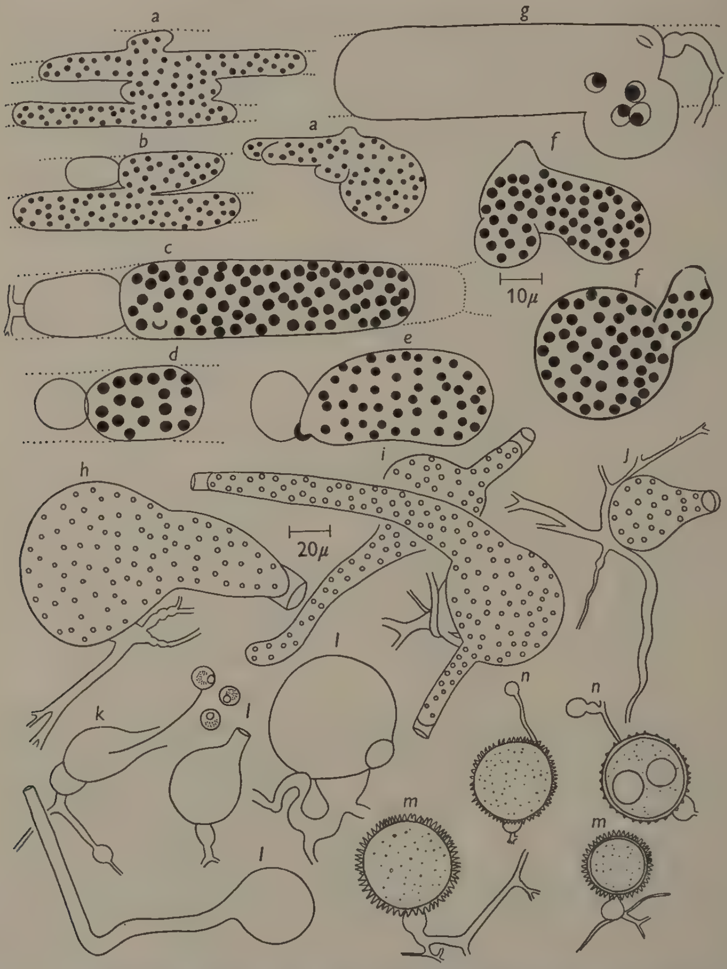
Entophlyctis sp. 2; *o*. Growth was apparently endobiotic. The persistent zoospore cyst (or possibly developing exit papilla) in young thalli was 3·5–4·5 μ across (Text-fig. 4*n*, *o*). At maturity, sporangia were ovoid, 15 × 11 μ, with bright orange zoospore globules 2 μ diam. (Text-fig. 4*p*). Dehiscence and resting spores not observed.

**Karlingiomyces marilandicus* (Karling) Sparrow (1960); *g*, *o*. Sporangia globose, 25–65 μ diam., oval 40–80 × 30–55 μ, obpyriform 70–80 × 40–55 μ, or oblong 70–80 × 25–40 μ (Text-fig. 4*f*–*j*). Rhizoids inserted at 2 or 3 points on the sporangium and deeply constricted into catenulations 7–20 μ long × 4–16 μ wide. A single prominent domed exo-operculum 8–12 μ diam., and exit pore 9–19 μ diam. (Text-fig. 4*i*, *j*). Zoospores spherical, 5 μ diam., with a hyaline lens-shaped globule 3 μ diam. and flagellum 30 μ long (Text-fig. 4*h*). Resting spores (not reported by Karling) globose 50 μ diam., with a brown wall 1 μ thick (Text-fig. 4*k*). There was general agreement with Karling's account, but sporangia with endo-opercula, prominent exit tubes, or a persistent zoospore cyst were not observed.



Text-fig. 5. Cellulosophilic species from the lake margin. *a*–*c*, *Entophlyctis* sp. 1; *d*–*f*, monocentric sp. 2. *a*, Young thalli with persistent zoospore cyst; *b*, mature sporangia with long exit tubes; *c*, a dehisced sporangium; *d*, surface view of a sporangium showing protuberances; *e*, optical section of a mature sporangium; *f*, an exit pore. All to the same scale.

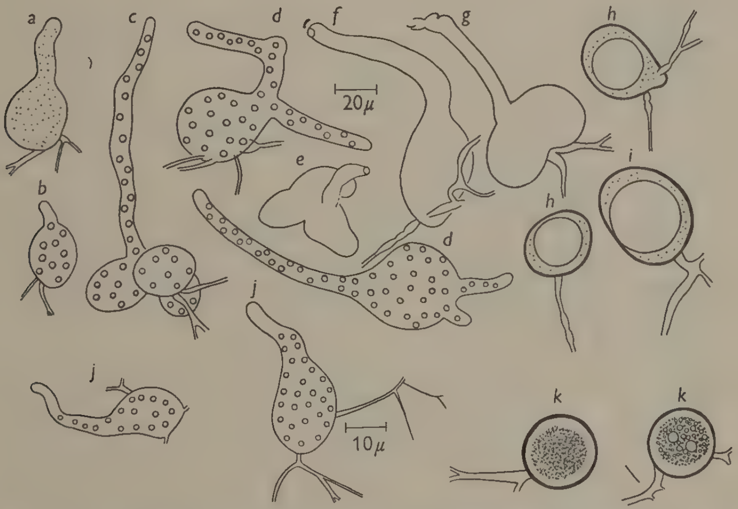
**Nephrochytrium aurantium* Whiffen (1941); *g*. Deeply lobed sporangia of this conspicuous pigmented chytrid often completely filled a number of adjacent grass cells (Text-fig. 6*a*–*d*) and extended up to 130 μ. Apophysis spherical, 5·5–15 μ diam., or oblong 17–27 × 12·5–15 μ. Zoospores spherical, 4·5–5 μ diam., with an orange globule 3 μ diam. Emergence of zoospores not seen, but it seems reasonably certain that the prominent domed structure (frequently thickened) on mature sporangia was an operculum (Text-fig. 6*c*, *e*, *f*). Although no persistent zoospore cyst was found, growth was endobiotic in all cases, and the general dimensions recorded



Text-fig. 6. Cellulosophilic species from lake mud. *a–g*, *Nephrochytrium aurantium*; *h–n*, *Diplophlyctis amazonense*. *a–b*, *h–j*, Mature sporangia; *b*, *c*, *d*, with apophyses; *e*, with apophysis and thickened operculum; *f*, with opercula; *g*, *k*, *l*, dehisced sporangia and zoospores; *m*, resting spore; *n*, resting spores with attached germ tube (?). In *a–d*, outlines of the grass cells in which the fungus is growing are represented by dotted lines. Scale for *a*, *b*, *h–j*, to right of *h*; all others to scale at right of *c*.

agreed well with Whiffen's account. It is possible that certain large thalli described as variants of *Cladochytrium replicatum* by Karling (1931) might be referred to this species.

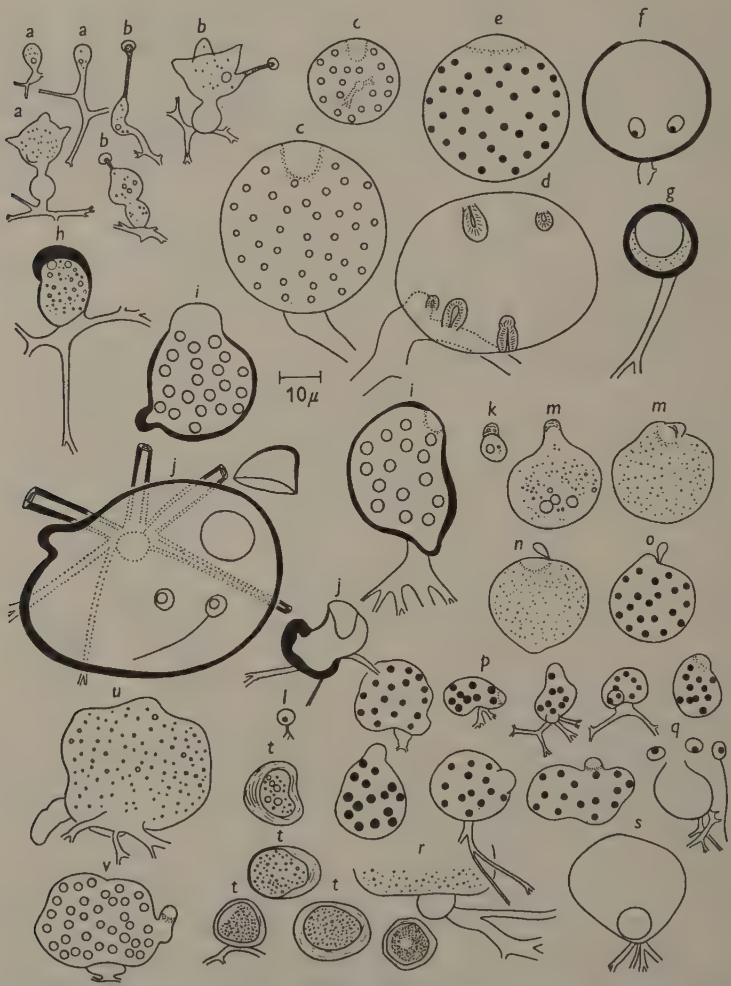
**NOWAKOWSKIELLA DELICA* Whiffen (1943); *o*. Polycentric, endobiotic, operculate. Sporangia globose, 20–75 μ diam., obpyriform 50 × 27 μ, or oblong 45–60 × 27–35 μ (Text-fig. 12*p–r*). Rhizoids predominantly tubular, frequently coiled or twisted, occasionally with aseptate swellings 8–14 μ diam. (Text-fig. 12*r*, *s*). At maturity both sporangium wall and zoospore globules (3·5–4 μ diam.) were sometimes light yellow, otherwise hyaline. Domed operculum 11–15 μ diam. (Text-fig. 12*q*). Exit pore 10–15 μ diam. Zoospores spherical, 6 μ diam. Resting spores spherical, 45 μ diam., with a hyaline wall 1 μ thick, and a single hyaline globule 35 μ diam. (Text-fig. 12*t*). The characteristic features were the conspicuous operculum, whorled rhizoids, and incurved septum cutting off the base of the mature sporangium (Text-fig. 12*p–r*). Whether or not the sterile portion so formed should be regarded as an apophysis is debatable, since it was not swollen laterally. Whiffen has described a similar structure in the material she studied, and in view of the absence of any other particularly distinctive characters, a placing in her species is thought to be justified.



Text-fig. 7. Cellulosophilic species from lake mud. *a–i*, *Endochytrium digitatum*; *j*, *k*, *Rhizophlyctis* sp. 1. *a*, Young sporangium; *b–d*, mature sporangia; *e*, dehisced sporangium; *f*, *g*, dehisced sporangia with detached opercula; *h*, *i*, resting spores; *j*, mature sporangia; *k*, resting spores. *a–h* to scale at right of *d*; *i–k* to scale at right of *j*.

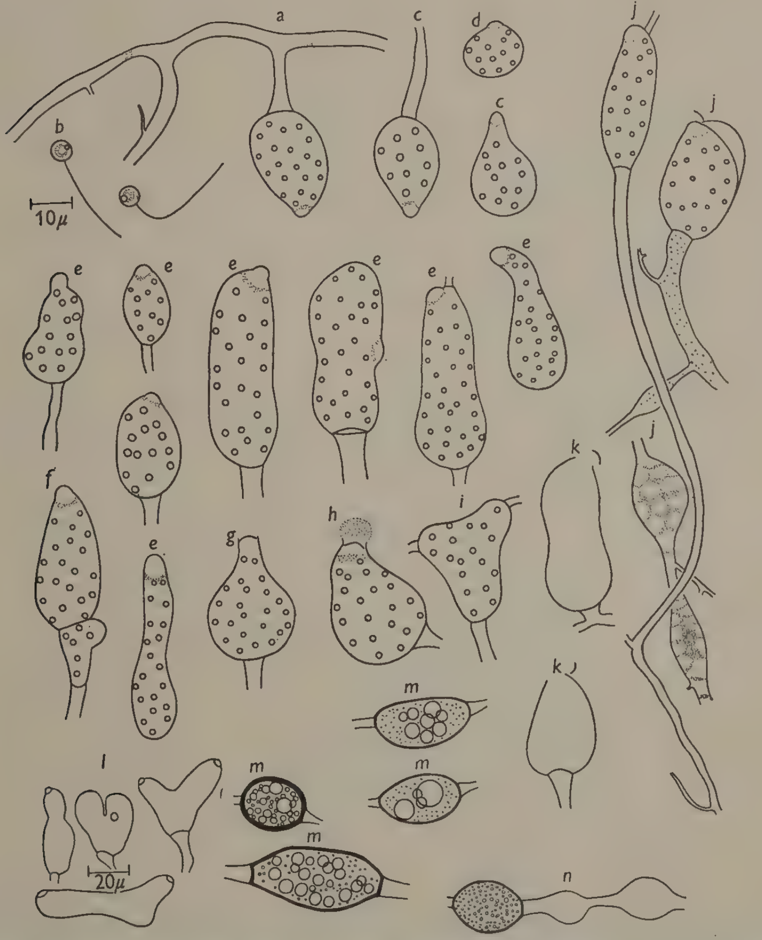
N. ELEGANS (Nowak.) Schroet. (Sparrow, 1960); *c*, *g*, *o*. Sporangia globose, 20–50 μ diam. (Text-fig. 13*a–c*); oblong specimens up to 95 μ long, filling the cells of the substratum tissue, seen only rarely (Text-fig. 13*d*). Apophysis 9–15 μ diam., exit pore 4·5–6·5 μ diam. Zoospores spherical 6·5–7·5 μ diam., with a conspicuous hyaline globule 2·5–4·5 μ diam. (Text-fig. 13*c*). Profuse development outwards from the substratum, often leading to the formation of a distinct growth fringe of virtually pure material. A study both of this and of pure single spore isolates has led to the view that the apophysis is a reasonably constant character, in contrast to Emerson's (1958) observations.

N. ELEGANS (non-apophysate); *g.* Sporangia globose, 16–32 μ diam., obpyriform 23–35 × 15–25 μ, oblong 21–75 × 12–30 μ, or cylindrical 42–80 × 8–25 μ (Text-fig. 12 *a–l*). Rhizoids up to 6 μ diam., often deeply constricted (but not septate), especially when passing through walls of the substratum tissue (Text-fig. 12 *a*, *j*, *m*; Pl. 22 *g*). At maturity sporangia



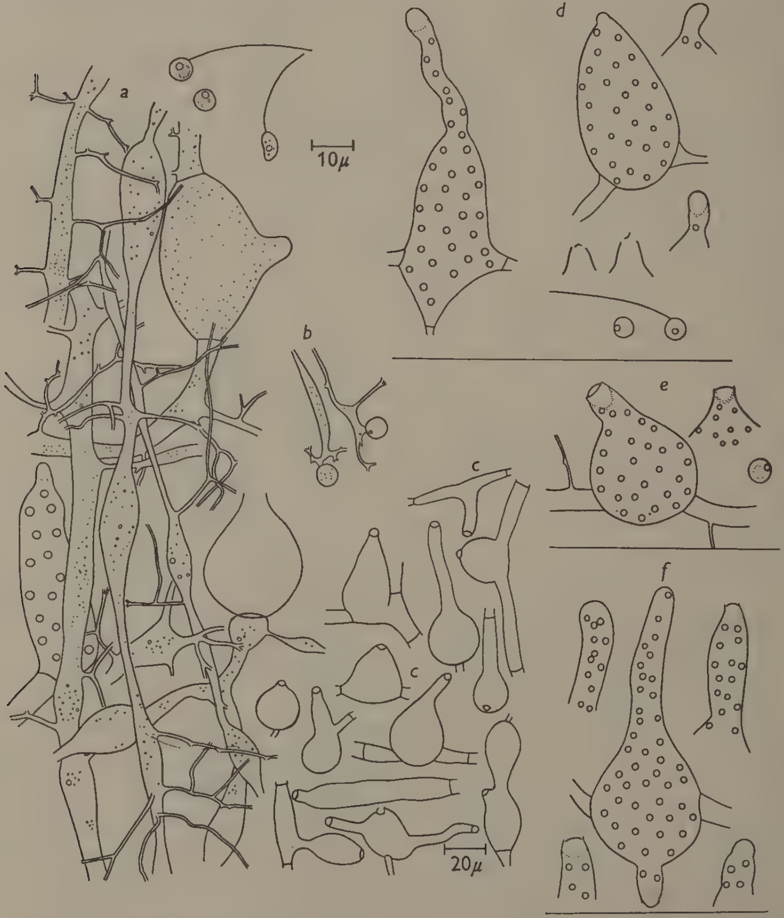
Text-fig. 8. Chitinophilic species from lake mud and from the lake margin. *a*, *b*, *Asterophlyctis sarcoptoides*; *c*, *d*, *Chytriomyces hyalinus*; *e–g*, *C. aureus*; *h–j*. *C. appendiculatus*; *k–t*, monocentric sp. 1; *u*, *v*, monocentric sp. 3. *a*, Young thalli showing direct development; *b*, with a persistent zoospore cyst showing indirect development; *c*, mature sporangia; *d*, dehisced sporangium with nodules; *e*, mature sporangium; *f*, dehisced sporangium with zoospores; *g*, resting spore; *h*, young thallus with unequally developed thickening; *i*, mature sporangia; *j*, dehisced sporangia; *k*, *m*, young pigmented thalli with a thickened persistent zoospore cyst; *l*, young thallus showing direct development; *n*, young thallus with an empty zoospore cyst; *o*, mature sporangium with an empty zoospore cyst; *p*, mature sporangia; *q*, dehisced sporangium with zoospores; *r*, sporangium base showing an apophysis; *s*, dehisced sporangium; *t*, resting spores; *u*, young thallus; *v*, mature sporangium. All drawings to the same scale.

contain spherical or lens-shaped hyaline globules 2·5–5·5 μ diam. Exit tubes, when present, 11–45 μ long × 4·5–13 μ wide. Operculum 3·5–7 μ diam., sometimes uniformly very thick (Text-fig. 12*b*, *k*) or with an upper (apparently gelatinous) zone in addition to the normal basal portion (Text-fig. 12*i*). Exit pore 3–9 μ diam. Zoospores 6–7·5 μ diam., with a



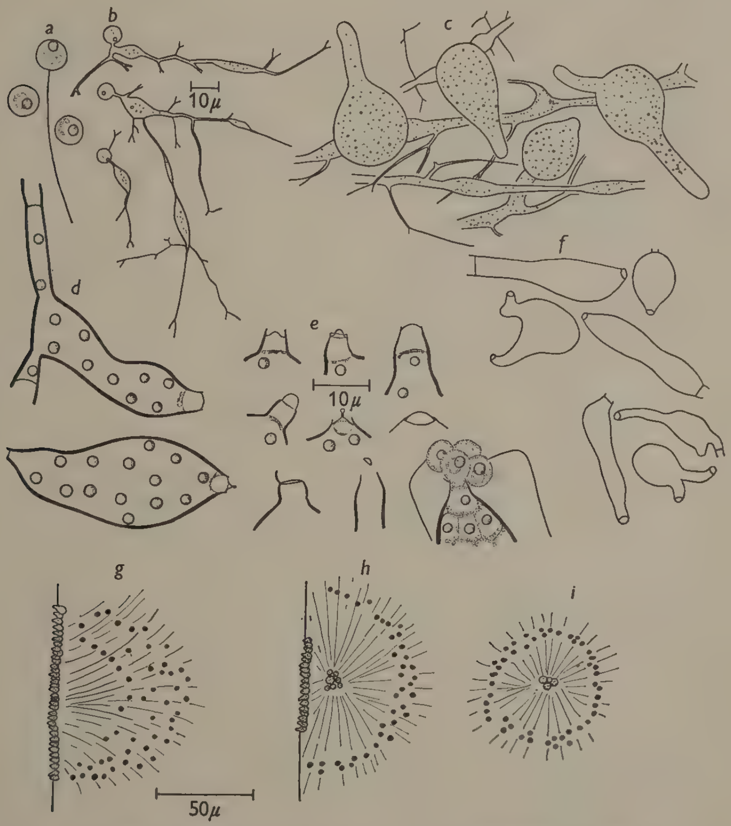
Text-fig. 9. Cellulosophilic species from lake mud. *Nowakowskiella profusa*. *a*, Mycelium bearing mature sporangium; *b*, zoospores; *c–e*, typical exo-operculate mature sporangia; *f*, two terminal sporangia; *g*, *h*, endo-operculate mature sporangia; *i*, an irregular mature sporangium; *j*, habit drawing showing mature sporangia and aseptate mycelial swellings; *k*, dehisced sporangia with opercula; *l*, range of form exhibited by irregularly shaped sporangia; *m*, resting spores; *n*, resting spore with adjacent aseptate mycelial swellings. Scale for all drawings, except *l*, below *b*.

spherical or lens-shaped hyaline globule, 3–6 μ diam. and flagellum 23 μ long, often amoeboid on emergence (Text-fig. 12*h–j*, *l*). At dehiscence zoospores were often compelled to squeeze through the narrow exit pore in order to emerge. Resting spores ovoid, 27–40 × 21–33 μ, or oblong 37–55 × 20–25 μ, with a brown wall 1 μ thick, and 1 or 2 yellow globules 16–25 μ diam. (Text-fig. 12*m*, *n*). This polycentric chytrid is clearly very similar to *N. elegans* as described above. However, a study of very abundant material suggests that it can be separated on a number of grounds and is regarded here as a variety of that species. An apophysis is never present, and in this it resembles *N. elongata* Karling (1944*a*). Again, in contrast to *N. elegans*, it has been recorded on a single cellulose-type bait only. Furthermore, when growing in grass leaves, the shape of the sporangium almost always depends on that of the confining cell, and dense extra-



Text-fig. 10. Cellulosophilic species from lake mud. *Nowakowskiella profusa.* Pure culture material. *a*, Habit drawing showing zoospores, sporangia, and mycelium with aseptate swellings and fine lateral branches; *b*, germination of zoospores; *c*, outlines of mature sporangia showing range of form; *d*, exo-operculate single spore isolate; *e*, endo-operculate single spore isolate; *f*, a single spore isolate with both types of operculation. All drawings, except *c*, to the same scale.

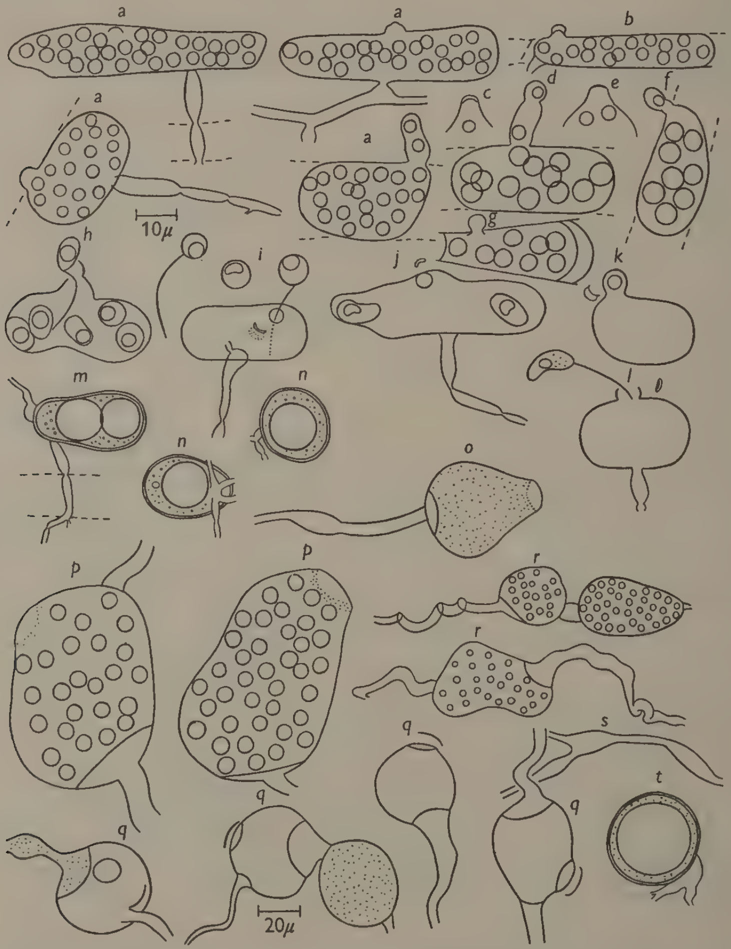
matrical growth fringes have never been observed. The peculiar spherical terminations to the exit tube (Text-fig. 12*d*, *f*, *g*, *k*) do not occur in typical material of *N. elegans*. The operculum varies in thickness, and probably in precise mode of development. On one occasion material was obtained in which an operculum was apparently absent, the exit pore being closed by a gelatinous plug (Text-fig. 13*e*), which eventually dissolved, leaving an open pore (Text-fig. 13*f*), though the actual emergence of the zoospores was not seen.



Text-fig. 11. Cellulosophilic species from lake mud. *Nowakowskiella profusa*. *a–f*, An atypical, pure, single spore isolate characterized by large zoospores; *g–i*, types of colony obtained on waterproof cellophane bait. *a*, Zoospores; *b*, germination of zoospores; *c*, mycelium and young sporangia; *d*, mature sporangia; *e*, opercula before and after dehiscence; *f*, mature sporangia showing range of form exhibited. In *g–i* sporangia are at the cellophane edge, at the cellophane edge and colony centre, and at the colony centre only, respectively. Radiating mycelium bearing resting spores is embedded in the substratum. *b*, *c* and *f* to scale below *b*; scale for other drawings, except *g–i*, below *e*; scale for *g–i* at right of *g*.

N. HEMISPHAEROSPORA Shanor (1942); *c*.

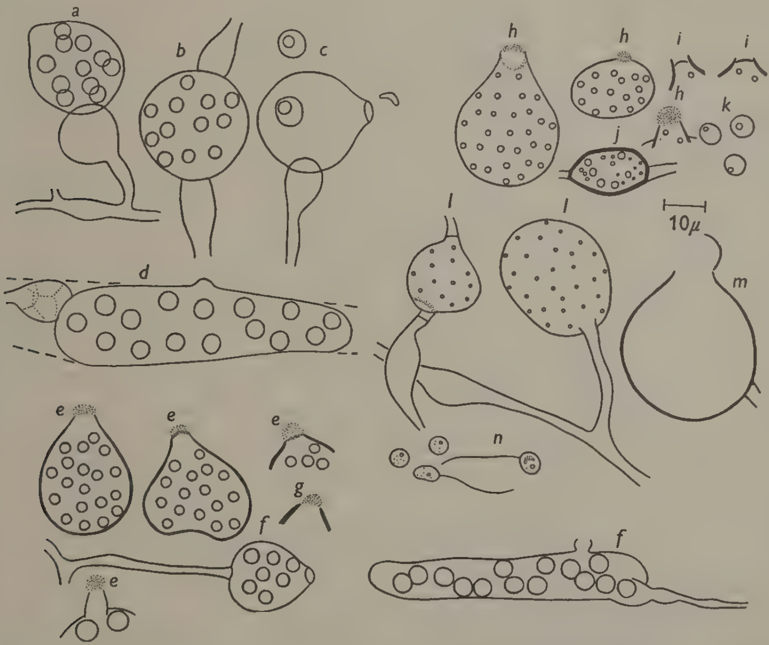
**N.* PROFUSA Karling (1941, 1944*a*); *c*, *g*, *o*. This polycentric species was obtained in great quantity, and other work suggests that it is widespread in the Lake District. Miss M. Roberts and R. N. de Fonseka (personal communications) indicate that it is probably equally common in the south of England, and since it is clearly an important aquatic species, a detailed account of material obtained both on baits and in pure culture is given.



Text-fig. 12. Cellulosophilic species from lake mud. *a–n*, *Nowakowskiella elegans* (non-apophysate); *o–t*, *N. delica*. *a*, *d*, *f*, *g*, Mature sporangia; *b*, mature sporangium with a thick operculum; *c*, *e*, opercula on mature sporangia; *h–l*, dehisced sporangia showing detached opercula and zoospores; *m*, *n*, resting spores; *o*, young sporangium; *p*, mature sporangia; *q*, dehisced sporangia showing detached opercula; *r*, *s*, details of rhizoids; *t*, resting spore. In *a*, *b*, *d*, *f*, and *m* the outlines of the grass cells in which the fungus is growing are represented by broken lines. Scale for *m–o*, *q*, *r–t* below *q*; all other drawings to scale below *a*.

On baits (Pl. 22 *a–d*) sporangia (Text-fig. 9 *c–e*) were globose 12·5–30 μ diam., pyriform 15–40 × 11–25 μ, or oblong 25–55 × 6–20 μ; sporangia of any other shape were rare (Text-fig. 9 *i*, *l*). Mycelium usually isodiametric, up to 7 μ wide, with occasional aseptate swellings, 10–27 μ long × 7–19 μ

wide (Text-fig. 9*a*, *j*, *n*). Operculum small but conspicuous, 4–5 μ diam., usually superficial (Text-fig. 9*e*, *j*, *k*), but very occasionally formed below a gelatinous plug which dissolved before dehiscence (Text-fig. 9*g*, *h*). Once, in material of the latter type, formation of the endo-operculum occurred so late in development that dehiscence was often inoperculate rather than operculate (Text-fig. 13*h*, *i*). Zoospores spherical, 4·5–5·5 μ diam., with a lateral hyaline globule 1–1·5 μ diam., and posterior flagellum 25 μ long (Text-fig. 9*b*). There was brief collective swarming before dispersal. Resting spores spherical, 13–17 μ diam., or oblong 17–35 × 7–15 μ, with a hyaline wall 0·5–1 μ thick and numerous hyaline oil globules (Text-fig. 9*m*, *n*). On both grass and cellophane dense growth fringes developing away from the substratum were frequently observed. Growth of colonies on waterproof cellophane followed such characteristic patterns that these are illustrated (Text-fig. 11*g*–*i*; Pl. 22*e*).



Text-fig. 13. Cellulosophilic species from lake mud. *a*–*d*, *Nowakowskiella elegans*; *e*–*g*, *N. elegans* (non-apophysate), illustrating inoperculate material; *h*–*k*, *N. profusa*, illustrating material with both operculate and inoperculate dehiscence; *l*–*n*, *Nowakowskiella* sp. 1. *a*, *b*, *d*, Mature sporangia; *c*, dehisced sporangium; *e*, mature sporangia with gelatinous plugs; *f*, mature sporangia with gelatinous plugs dissolved; *g*, dehisced sporangium with gelatinous plug still in position; *h*, mature sporangia with gelatinous plugs; *i*, endo-opercula; *j*, resting spore; *k*, zoospores; *l*, mature sporangia and rhizoids; *m*, dehisced sporangium; *n*, zoospores. In *d*, the outline of the grass cell in which the fungus is growing is represented by broken lines. All drawings to the same scale.

Pure cultures were made on 14 strength YpSs agar, and sporangium production was good in a liquid medium of the same composition plus 0·5 % glucose and 0·1 % casamino acids. In pure culture sporangia were rather variable in shape, globose 15–43 μ diam., ovoid 30–50 × 15–32 μ, pyriform 18–80 × 10–47 μ, spathulate 35–45 × 15–20 μ, oblong 40–75 × 11–25 μ, or irregular (Text-figs. 10*c*; 11*f*). Extension of the sporangium laterally into adjacent portions of the mycelium (Text-figs. 10*c*; 11*d*), and the development of prominent exit tubes were cultural characters unknown on baits. Exit tubes were up to 50 μ long and 3–10 μ wide (Text-figs. 10*d*,*f*, 11*c*;*f*; Pl. 23, *h*–*j*). Details of the mycelium and zoospores were as described above, but the improved conditions of culture made it possible to observe many fine branches from the main rhizoidal axes (Text-figs. 10*a*; 11*c*; Pl. 23*h*, *i*). Resting spores did not develop in culture. Of the single spore isolates studied, some were consistently exo-operculate (Text-fig. 10*d*), others consistently endo-operculate (Text-fig. 10*e*), while others were of both types (Text-fig. 10*f*). In all the material both from pure culture and from bait, endo-opercula were never deep-seated, but always lodged just below the exit pore tip. One single spore isolate (Text-fig. 11*a*–*f*) was characterized by large (6–7 μ diam.) zoospores, but otherwise agreed fairly well with the other isolates. From Karling's description (1949*a*), *N. crassa* appears almost identical with *N. profusa*, and it seems very possible that they form a single species.

NOWAKOWSKIELLA sp. 1; *o*. Polycentric, endobiotic, operculate, with globose sporangia, 17–32 μ diam. and rhizoids with aseptate swellings up to 8 μ across (Text-fig. 13*l*); at maturity sporangium walls were light brown. Operculum 5–10 μ diam. (Text-fig. 13*m*). Zoospores spherical 4·5 μ diam., with a lateral hyaline globule 0·7 μ diam. and posterior flagellum 20 μ long (Text-fig. 13*n*). The minute zoospore globule was the most striking feature of this chytrid, which cannot readily be placed in any described species.

**PHLYCTOCHYTRIUM IRREGULARE* Koch (1957); *g*, *o*. Sporangia globose 42–50 μ diam., subglobose 20–50 × 17–50 μ, ellipsoid 17–27 × 12–20 μ, obpyriform 15–50 × 10–30 μ, or oblong 37–65 × 20–35 μ (Text-fig. 1*j*–*m*). The conspicuous dehiscence papilla was 4–15 μ across. Two papillae were seen on a single specimen only (Text-fig. 1*k*). Exit tubes, when present, were 7·5–15 μ long and 6–7·5 μ wide. Zoospores spherical, 3·5–4·5 μ diam., or ovoid 4–5 × 3–4 μ, with a hyaline globule 1–2 μ diam. and posterior flagellum 24–26 μ long. Resting spores not observed. Dehiscence was usually inoperculate (Text-fig. 1*l*), but structures resembling opercula or fragments of these were seen occasionally at the exit pore rim (Text-fig. 1*m*). These fragments, often recurved, suggested that incomplete liquefaction of the boundary membrane of the papilla apex had occurred. Although neither an apophysis nor punctate markings on the sporangium wall were seen in the Esthwaite material, the general resemblance to the chytrid described by Koch is so close that a placing in his species is considered appropriate.

P. PLANICORNE Atkinson (1909); *c*, *g*, *o*. Sporangia spherical, 10–25 μ diam, with an apophysis 4·5–11 μ diam. Zoospore globules 1–1·5 μ diam. Apical teeth (4, 6, 8 or 9) 2·5–5 μ long (Text-fig. 4*a*–*d*; Pl. 22*f*). Zoospores not observed.

PHLYCTORHIZA VARIABILIS Karling (1947*b*); *n*. This proved to be a most difficult chytrid to study, due partly to the fact that most thalli developed indirectly from the zoospore cyst (Text-fig. 3*a*, *b*, *d*, *g–i*, *k*) and at maturity were embedded in the substratum. Since a placing in Karling's species cannot be made with absolute confidence, some details are given. The persistent zoospore cyst was 3–4 μ diam. and the germ tube which developed from it 3–35 μ long and 3·5–12·5 μ wide. Sporangia were 25–65 μ across and the exit pore (usually single) 3·5–12 μ diam. (Text-fig. 3*c–g*). Resting spores were 15–45 μ diam. with a brown wall 1–1·5 μ thick (Text-fig. 3*h–j*). Although enormous numbers of thalli were available for study, a remarkable feature was the difficulty experienced in inducing dehisence of mature sporangia. Zoospores seen on one occasion only were spherical 2–2·2 μ diam., with granular contents. There is thus a discrepancy between recorded sizes of zoospores and zoospore cysts. Groups of thalli (e.g. Text-fig. 3*k*) were very characteristic (but not reported by Karling) and were interpreted as the result of germination of zoospores in situ in dehisced sporangia.

RHIZIDIUM RICHMONDENSE Willoughby (1956); *c*, *g*, *o*. Most specimens agreed well with the material previously described, e.g. Text-fig. 1*e*. In addition, a number of deeply lobed sporangia were seen (Text-fig. 1*f*), and some in which growth at the apex had partly (Text-fig. 1*h*) or completely (Text-fig. 1*g*), obliterated the zoospore cyst outline.

**RHIZOPHLYCTIS CHITINOPHILA* (Karling) Sparrow (1960); *s*.

R. ROSEA (de Bary & Woronin) Fischer (Johanson, 1944); *c*, *g*, *o*. There was good agreement with previous British material (Willoughby, 1958), except that thalli with a germ tube or ornamentation were not found. Pigmentation was rather variable, many orange specimens being seen.

**R. ROSEA* (polycentric); *c*. Polycentric, endo-operculate, with orange sporangia 20–105 μ across. Exit tubes 10–35 μ long with exit pores 3·5–10 μ diam. Large sterile swellings on the rhizomycelium developed consistently (Text-fig. 4*e*). Zoospores spherical, 5 μ diam., with granular contents. Since associated monocentric thalli were not seen, this chytrid appears to be quite distinct from *R. rosea* (monocentric) discussed above. It is noted, however, that Karling (1947*a*) reported limited polycentric tendencies in typical monocentric material of *R. rosea*. The Esthwaite chytrid cannot readily be referred to *Catenomyces persicinus* Hanson (1945), which it resembles, since there were no apiculate or spined endo-opercula.

RHIZOPHYDIUM ELYENSIS Sparrow (1957); *g*, *n*. On snake skin sporangia were polygonal at maturity, mostly 15–35 μ diam., but also up to 55 μ, with 5–8 exit pores, 4–8 μ diam. On grass sporangia were mostly 20–30 μ diam., though some were 8–60 μ; 5–9 exit pores, 4–7·5 μ diam. (Text-fig. 1*a*, *b*). On both types of substratum there was a single delicate branched rhizoidal axis, very difficult to observe; zoospores were spherical, 2·5–3 μ diam., with a small hyaline globule 0·5–1 μ diam., and flagellum 15 μ long (Text-fig. 1*d*). Resting spores not observed. Separate records have been kept of the thalli on snake skin and on grass leaves, since it is unusual for a single species to grow both on keratin and on cellulose-type baits. However, the two could not be distinguished on morphological grounds, and cross-inoculation and nutritional studies would be most interesting. Previous work (unpublished) in the south of England indicates that this species is widespread and a very typical soil form.

R. KERATINOPHILUM Karling (1946); *n.*

R STIPITATUM Sparrow (1957); *c, t.* Only very limited material was available and the naming is tentative. Sporangia spherical, 35–45 μ diam., with a single rhizoid, the main axis of which extended up to 50 μ before branching. The 2–3 prominent dehiscence papillae were 3–6 μ tall × 9–20 μ wide. Zoospore globules hyaline, 2–3 μ diam. Zoospores and resting spores not observed.

Rhizophlyctis ingoldii Sparrow (1957); *n, s, t.* Growth was prolific on chitin baits, but very few thalli were seen on snake skin. Sporangia globose, subglobose, ellipsoidal, or ovoid, 15–125 μ long × 10–125 μ wide, with a single large dehiscence papilla; bearing a number of separate coarse rhizoidal axes up to 29 μ diam. (Text-fig. 2*a, c, e, f*) or, more rarely, a single large one (Text-fig. 2*b, d*). Dehiscence papilla up to 10 μ tall and 8–35 μ wide, with a conspicuous zone of clear cytoplasm 9–25 μ deep. Dehiscence inoperculate (Text-fig. 2*g, h*), leaving an exit pore 9–37 μ diam., rim smooth (Text-fig. 2*j*) or irregular (Text-fig. 2*i, k*). Zoospores spherical, 4·5–5·5 μ diam., with a hyaline globule 1·5–2 μ diam. situated near the flagellum insertion; posterior flagellum 22–26 μ long (Text-fig. 2*n*). Resting spores (not reported by Sparrow) spherical, 10–32 μ diam., with a yellow wall 1–1·5 μ thick and a single grossly thickened rhizoidal axis (Text-fig. 2*l*), or a number of more normal separate ones (Text-fig. 2*m*). An ornamentation of branched hairs projecting up to 25 μ occurred consistently on both sporangia and resting spores (Text-fig. 2*a, b, l, m*). It seems very possible, however, that this ornamentation was an epiphytic growth of associated micro-organisms, such as actinomycetes. Although there are some differences of detail from Sparrow's account, the unusually large dehiscence papilla is clearly the most significant character, and the shape and size of this agreed well with his description.

Since the above account was written, it has been possible to obtain a soil sample from 17 Barton Road, Cambridge, one of Sparrow's cited localities for *R. ingoldii*. The chytrid obtained in great abundance when chitin baits were used with this sample is clearly the same species as that described from Esthwaite. In the Cambridge material the sporangia were not ornamented with branched hairs, but at the same time the granular incrustation described by Sparrow was not clearly distinguished, and it seems very possible that this too can be ascribed to the activity of associated micro-organisms. Details of dehiscence papillae and exit pores agreed well with those given above for the Esthwaite material, though zoospore globules were rather smaller (0·5–1·5 μ diam.) but within the range obtained from collections of *R. ingoldii* from the whole Lake District. These other collections will be described later. Resting spores were not seen in the Cambridge material.

Rhizophlyctis sp. 1; *o.* Sporangia obpyriform, 17–25 × 10–15 μ, with 2–3 rhizoidal axes and a long exit tube (Text-fig. 7*j*); exit tubes 17–22 μ long and 3·5–4 μ wide. Zoospore globules hyaline, 1 μ diam. Resting spores spherical, 16–18 μ diam., with a brown wall 0·5 μ thick (Text-fig. 7*k*).

Rhizophlyctis sp. 2; *o.* Sporangia globose, 45 μ diam., with 1 or 2 projecting exit papillae and several separate rhizoidal axes (Text-fig. 4*l, m*), inoperculate. Zoospores spherical, 4·5–5 μ diam., with a hyaline globule 1 μ diam. (Text-fig. 4*m*).

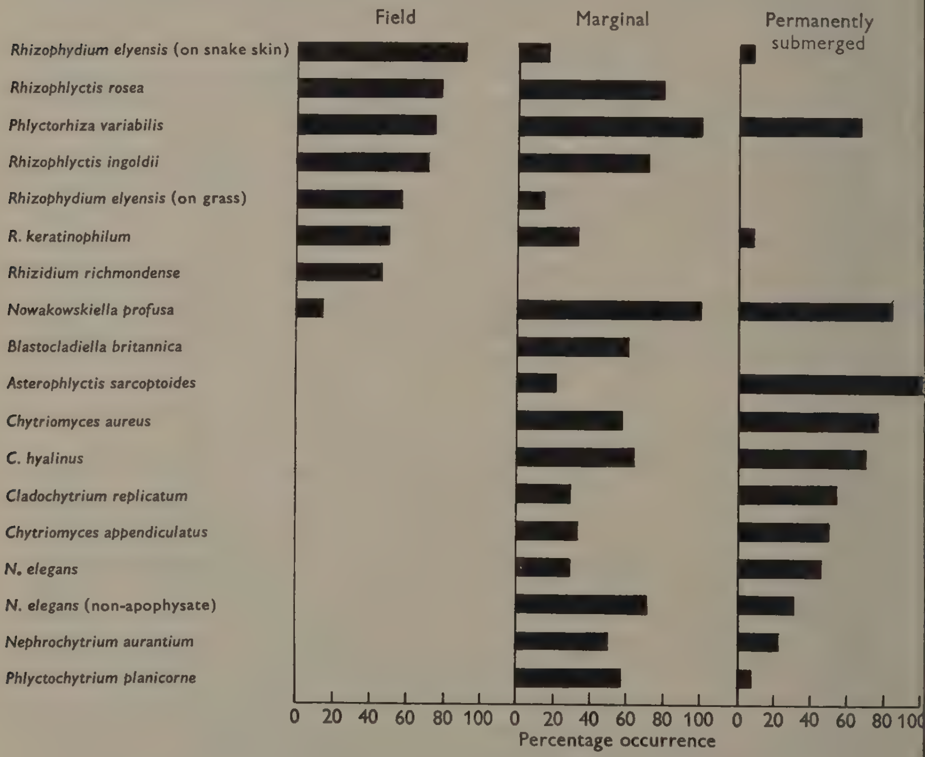
MONOCENTRIC sp. 1; *s*. Sporangia rather variable in shape (Text-fig. 8*o*, *p*, *q*, *s*), but always small (10–45 μ across). Usually a single rhizoidal axis. Apophysis, when present, 3–8·5 μ across. Dehiscence mechanism unknown. Dehiscence pore 4–7·5 μ diam. Zoospores oval 4–4·5 × 3–3·5 μ, with an orange globule 1·5–2 μ diam. and flagellum 22 μ long (Text-fig. 8*q*). Resting spores spherical, 11–22 μ diam., or oval up to 22 μ long, with an unequally developed brown layered wall up to 3 μ thick (Text-fig. 8*t*). Although it is possible that there are two species here, a study of young thalli indicated that development was either direct (Text-fig. 8*l*) or indirect (Text-fig. 8*k*, *m–o*), when the persistent zoospore cyst, which was either thickened or empty, was near the sporangium apex.

MONOCENTRIC sp. 2; *g*. Sporangia spherical, 17–27 μ diam., covered with tiny papilla-like protuberances 1·5–2 μ tall × 2–2·5 μ wide. Zoospore globules minute and hyaline, 0·5 μ diam. A number of exit pores (Text-fig. 5*d–f*).

MONOCENTRIC sp. 3; *s*. Sporangia 20–35 μ across, angular, with a prominent apophysis and exit tube. Apophysis 3·5–10 μ diam. Exit tube 5–15 μ long and 3·5–6 μ wide, usually lateral but occasionally terminal (Text-fig. 8*u*, *v*). Zoospore globule hyaline, 2–2·5 μ diam.

RESULTS OBTAINED WITH BAITING PROCEDURES

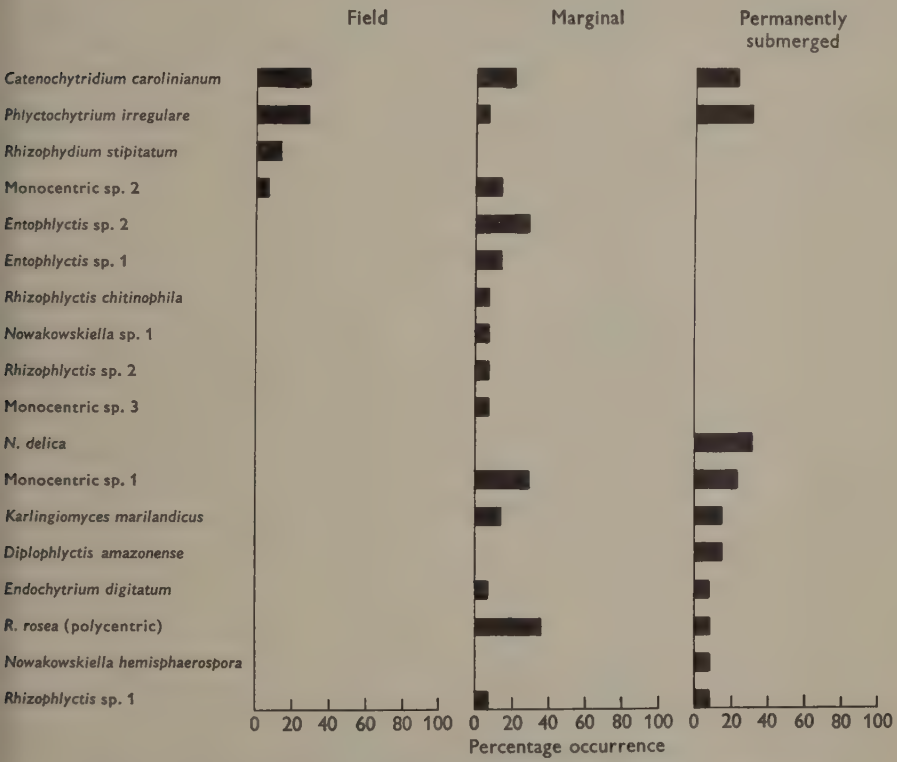
No attempt was made to estimate the number of thalli present on the baits, the aim being to obtain a comprehensive list of species recovered from each collection. For any one collection, therefore, each species was counted as present or absent. From each zone 14 collections were made and on this the calculated percentage occurrence of each species in each zone is based. Where a species was recovered from 3 collections, for example, the calculated percentage occurrence was 3/14 × 100 = 21. A record of 'present' might refer to a single thallus or to many thousands, and this could give misleading results. However, it was found that the distribution of most species was so strictly circumscribed that this was not a complicating factor. The species for which a high percentage occurrence was recorded were generally represented by very abundant material whenever found. On the other hand, species for which only low percentage occurrences were recorded were often represented by very meagre material. Since the examination of abundant material was usually an essential prerequisite to definite identification, it will be appreciated that a number of species, for which low percentage occurrences were recorded, are incompletely known. It was convenient to segregate these forms, by purely arbitrary means, so that special consideration could be given to the species which were very common, all included in Text-fig. 14 and having a percentage occurrence of more than 40 for any one zone. The only species with a high percentage occurrence from all three zones was *Phlyctorhiza variabilis*; but it is necessary to mention that only very few thalli were ever obtained from submerged mud collections, while enormous numbers were always obtained from field collections. The other species recorded, with the exception of *Blastocladiella britannica*, can clearly be regarded as either essentially terrestrial or essentially aquatic. It was noteworthy that all except one of the 18 species were obtained from the marginal zone, which indicates that this situation is a very favourable one for the growth of saprophytic chytrids. Of the 18 species for which 40 % occurrences were not obtained (Text-fig. 15), a similar pattern emerges; again the largest number of species was from the marginal zone.



Text-fig. 14. The distribution pattern of species for which a percentage occurrence of 40 or more was recorded in any one zone.

Of the 36 species on both lists, only 12 were obtained from field collections, in contrast to 31 from the margin, and 23 from the permanently submerged mud. Again, a number of forms so incompletely known that they are not included in Text-fig. 15 were obtained in this investigation, and all were recovered from marginal or permanently submerged mud collections. The final over-all impression obtained was that the field population differed from that of the other two zones in that it consisted of a distinctly limited number of species, most of which could be obtained in quantity very readily. In relation to the field population, the results obtained by Gaertner (1954) are of interest. On baiting 287 African soil samples for saprophytic chytrids he obtained *Rhizophlyctis rosea* from 217 and *Phlyctorhiza variabilis* from 45.

For the accumulation of evidence of seasonal periodicity at Esthwaite only the most common and abundant species in each zone were considered; those with a 70% or more occurrence are listed in Table 1. Presence or absence for each month is indicated. Of the instances of a species not being recorded in three or more consecutive months, two are of *Rhizophlyctis ingoldii* and one *Chytriomyces hyalinus*. In view of the discussion of *C. aureus* and *C. hyalinus* above, no particular significance is attached to these records for the latter species. However, since *R. ingoldii* was such a distinct specific entity, the records for this merit closer attention. The failure to obtain it from field collections in May–August inclusive was striking, since it was so abundant in the other months. Furthermore, additional field collections in May and June 1960 also failed to yield the fungus. It must be concluded, therefore, that the observed periodicity for field collections was probably valid, and the fact that the field was limed in early summer in both years may provide an explanation.



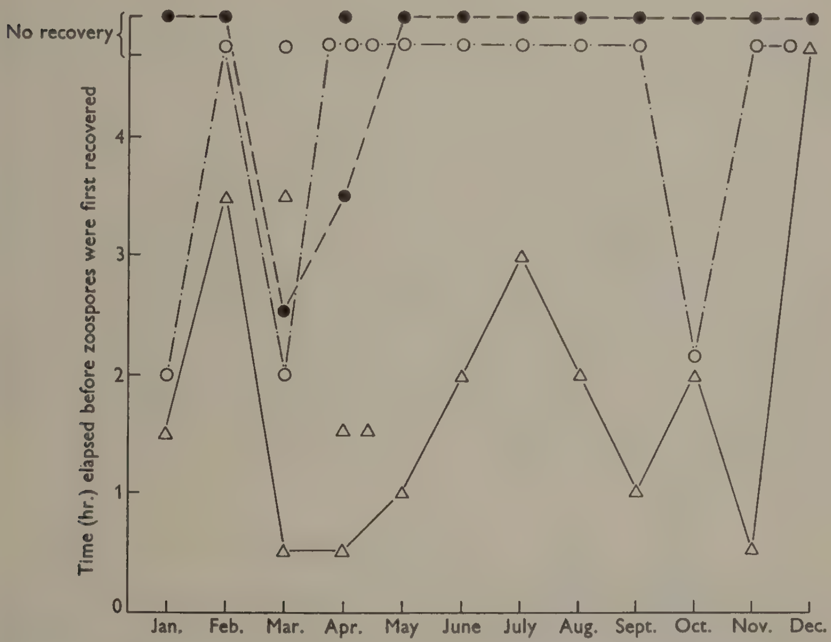
Text-fig. 15. The distribution pattern of species with a percentage occurrence lower than 40 in all zones.

Table 1. *Monthly records of the most common and abundant species at Esthwaite in* 1958

		Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Field	*Rhizophlyctis rosea*	+	−	+	+	−	+	+	−	+	+	+	+
	Phlyctorhiza variabilis	+	+	−	+	+	+	+	−	+	−	+	+
	Rhizophydium elyensis (on snake skin)	+	+	−	+	+	+	+	+	+	+	+	+
	Rhizophlyctis ingoldii	+	+	+	+	−	−	−	−	+	+	+	+
Marginal	*R. rosea*	−	+	+	+	+	+	+	−	+	−	+	+
	Nowakowskiella profusa	+	+	+	+	+	+	+	+	+	+	+	+
	N. elegans (non-apophysate)	+	+	−	+	+	+	+	−	−	+	+	+
	P. variabilis	+	+	+	+	+	+	+	+	+	+	+	+
	R. ingoldii	+	+	+	+	+	+	+	−	+	−	−	−
Permanently submerged	*Asterophlyctis sarcoptoides*	+	+	+	+	+	+	+	+	+	+	+	+
	Chytriomyces aureus	+	−	+	−	+	+	+	+	+	+	+	+
	C. hyalinus	−	−	−	−	+	+	+	+	+	+	+	+
	N. profusa	−	+	+	+	+	+	+	−	+	+	+	+

ZOOSPORE RECOVERY FROM SOIL AND MUD COLLECTIONS

A very limited amount of further information was obtained by a more direct method. In this connexion it must be admitted that the microscopic examination of soil or mud in order to find saprophytic chytrids has so far yielded nothing worthy of record. It became evident, however, that when soil or mud collections were placed in water and baited, young thalli often became established on the baits within a few hours. These observations prompted a more detailed investigation, since they suggested that mature sporangia were present in the actual collection, and that these produced zoospores after a short period in the laboratory. In this work on zoospore recovery from soil or mud collections the procedure adopted was to place 6 g. samples in small beakers (30 ml.) and just cover them with sterile charcoal water. Surface loopings of the water were taken at half-hourly intervals and searched immediately under the microscope for chytrid zoospores. The latter are aerotactic and tend to rise to the surface meniscus of water, and since they have such a characteristic appearance and type of movement, it was possible to recognize and recover them in this way. The first experiments were made in March 1958. With the beakers held in water baths at 5° and 25° zoospores were recovered from a marginal collection in 3·5 and 0·5 hr., respectively, and from a field collection in 7·5 and 2 hr., respectively. Since raising the temperature



Text-fig. 16. Zoospore recovery from mud and soil samples held at 25° C. Collections: ·○·—·○·, field zone; △—△, marginal zone; ●– –●, permanently submerged zone.

clearly accelerated recovery, all subsequent work was carried out at 25°. For each sample tested the time elapsing before zoospores were first recovered is recorded in Text-fig. 16. Zoospores were recovered from marginal zone samples in every month except one, but there were only two and three recoveries, respectively, from each of the other two zones. Particular interest is attached to zoospore recovery from the field zone in January, March and October, in view of the fact that so little information is available on the activity of aquatic phycomycetes in soil.

It seems scarcely possible that the zoospores obtained in this work could have been derived from germinated resting spores, since the times involved are so short. Resting spore germination in chytrids normally involves breaking of dormancy, re-organization of cytoplasmic contents, formation of a sporangium, nuclear division, delimitation of zoospores, and finally dehiscence; and the scant information so far available indicates that some days, rather than hours, are necessary for all these processes to be completed. It seems logical to suppose, therefore, that whenever zoospores were recovered they emanated directly from mature sporangia rather than indirectly from resting spores. It is thought to be significant that for any one collection zoospores were never recovered immediately, or recovered at all once 3·5 hr. had elapsed, and the observations were often carried out for several hours beyond the period indicated in Text-fig. 16. In order to explain these results, it is suggested that zoospore recovery was dependent on the mass dehiscence of mature sporangia in the sample, and this was induced rapidly (but not immediately) by flooding with fresh sterile water. If this explanation is correct, then the presence of mature sporangia in freshly collected samples almost certainly implies that active growth was proceeding in nature, and the fungi concerned were not merely present in resting stages.

PHYSICAL AND CHEMICAL CHARACTERISTICS OF THE ENVIRONMENTS INVESTIGATED

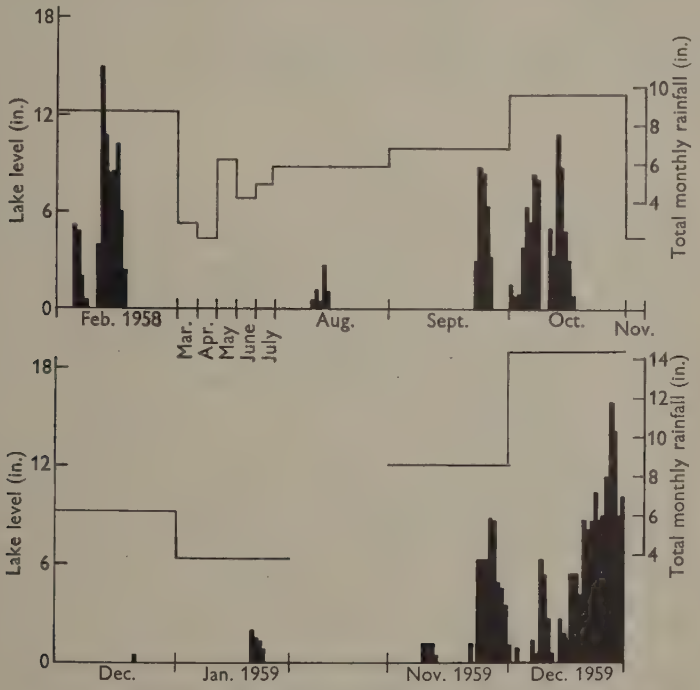
During the course of these investigations a number of physical and chemical analyses of the soil and mud were made (Table 2). Collections for this purpose were made in precisely the same way as for baiting. Determinations of moisture content were made by air drying to 105° and of organic matter content by igniting to 550°, for 6 hr.; pH values were made on

Table 2. *Physical and chemical analyses of soil and mud collections*

	Field	Marginal	Permanently submerged
Moisture content (% wet weight)	49·4–60·2 (mean 54·1)	74·4–81·9 (mean 78)	15·1–23·8 (mean 18·1)
Organic matter content (% dry weight)	21·3–30·9 (mean 24·6)	65·9–77·3 (mean 69)	70·3–85·7 (mean 81)
pH	5·7–6	6–7	6·2–6·4
Ca, m-equiv./100 g.	4·99	24·96	5·39
Na, m-equiv./100 g.	0·16	0·48	0·18
K, m-equiv./100 g.	0·18	0·87	0·17
PO_4P, m-equiv./100 g.	0·015	0·79	0·016

fourfold dilutions of the collection, using a glass electrode pH meter. Methods for the determination of exchangeable cations followed those of Pigott (1958).

The high moisture, organic matter, and exchangeable cation content of the marginal mud collections was most striking. There was a considerable difference in organic matter content between the field and permanently submerged zones. Permanently submerged mud was fine grained with no crumb structure. Despite its high organic matter content, microscopic examination never revealed recognisable fungal structures.



Text-fig. 17. Esthwaite records of lake level (daily) during flood periods, and of rainfall (monthly).

Analytical data were also obtained from Esthwaite water samples. At the usual collecting site the pH was 6·8 immediately above the mud and 7·2 at the surface. Estimations for Ca, Mg, Na, K, and PO_4P, all expressed as milliequivalents per 100 ml., were 0·04, 0·012, 0·02, 0·002 and 1–80 × 10^{-6}, respectively. In degree of hardness (Windle Taylor, 1958) the water can be placed in the soft category, though studies carried out by the Freshwater Biological Association indicate that the lake as a whole is highly productive in comparison with most others in the English Lake District.

LAKE LEVELS AND RAINFALL

During February 1958–January 1959, inclusive, and in November and December 1959, when additional collections were made, lake levels at Esthwaite were obtained daily. In Text-fig. 17 the abscissa 0 in. represents a lake level of 214·26 ft. above sea level, a level just sufficient to immerse completely the marginal zone; only lake levels above this appear in the figure and these occurred on 45 days during February 1958–January 1959, when the marginal zone was under water. The maximum water cover recorded was 15 in. in February, and the lowest recorded level, 11·5 in. below the 214·26 ft. O.D., was in early September. During the year total rainfall was 63·4 in. Marginal collections were made from under water in February 1958 and December 1959, the latter being an exceptionally wet month with a rainfall of 14·4 in.

DISCUSSION

The most striking feature of this investigation has been the demonstration that the marginal zone of this lake was an exceptionally favourable site for the growth of saprophytic chytrids. Evidence for this was seen not only in the large number of species recorded from marginal mud, but also in the ease with which zoospores were recovered from it and the rapidity with which added baits were decomposed by it. It is not known whether the particular topographical and climatic conditions which prevail at Esthwaite and which cause rapid fluctuations in lake level are largely responsible for the result obtained, or whether the margins of water bodies in general are characterized by a similar rich chytrid microflora. Comparison of the chytrid populations of the field and permanently submerged zones showed distinct differences in species composition, despite the fact that some species were common to both zones. The fact that certain species were obtained from soil but never from permanently submerged mud is particularly interesting, in view of discussions as to whether aquatic fungi recovered from soil by baiting methods constitute part of the active soil microflora, or whether they are present in that situation merely as resting spores blown or carried in from some adjacent water body. The evidence from Esthwaite is that these strictly confined terrestrial species constitute an active and endemic part of the soil microflora, since it does not seem feasible to regard them as 'contaminants' from the adjacent water body.

My thanks are due to my colleague, J. Heron, who kindly provided water analysis data, and also to L. W. Clayton who collected the Cambridge soil sample.

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EXPLANATION OF PLATES 22 AND 23

Plate 22

Material obtained by baiting with grass leaves (*a–d, f, g*) and with cellophane (*e*)

a–e, Nowakowskiella profusa; *f, Phlyctochytrium planicorne*; *g, N. elegans* (non-apophysate). *a, c, f, g,* mature sporangia, × 800; *b*, mature sporangia and resting spore, × 800; *d*, dehiscing sporangium, × 780; *e*, resting spores, × 400.

Plate 23

Pure culture material of *N. profusa* growing on YpSs agar, × 400. *h, i*, Young sporangia with exit tubes; *j*, dehiscing sporangium; *k*, early development from the zoospore (still recognizable—top).

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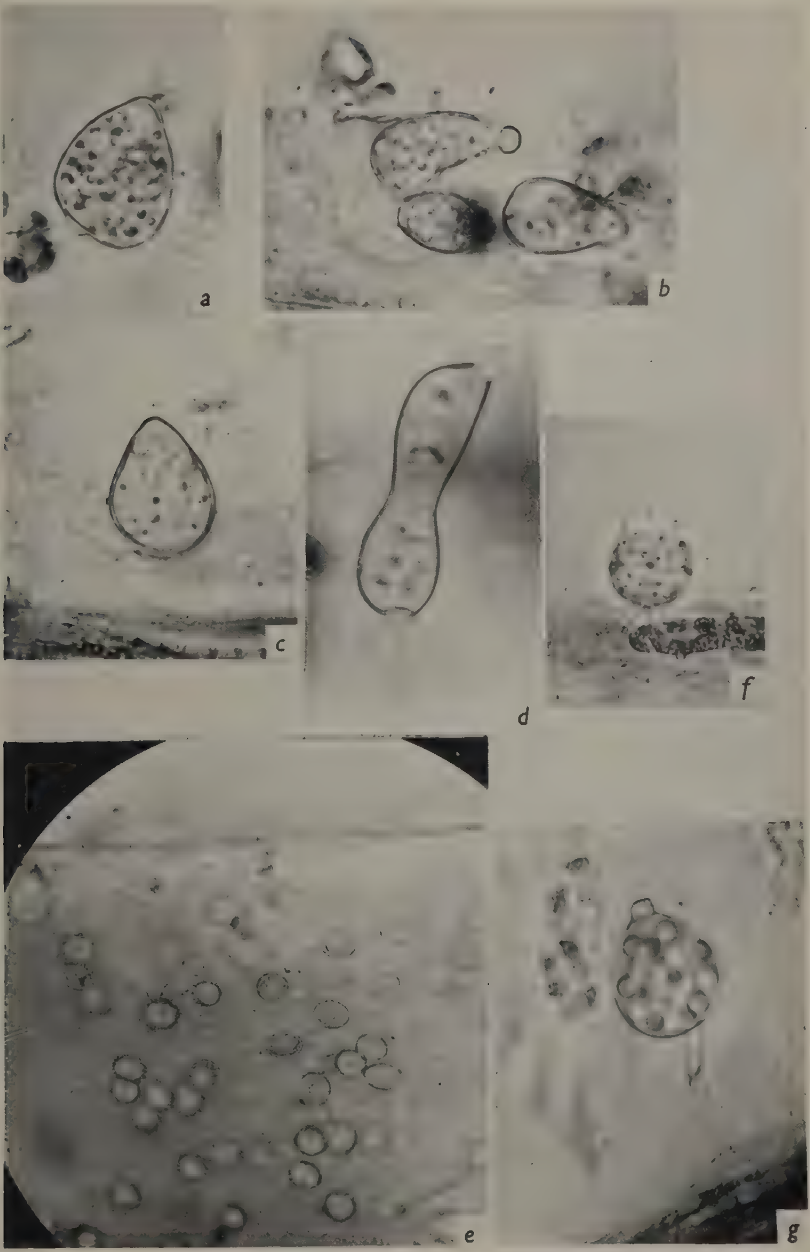
a
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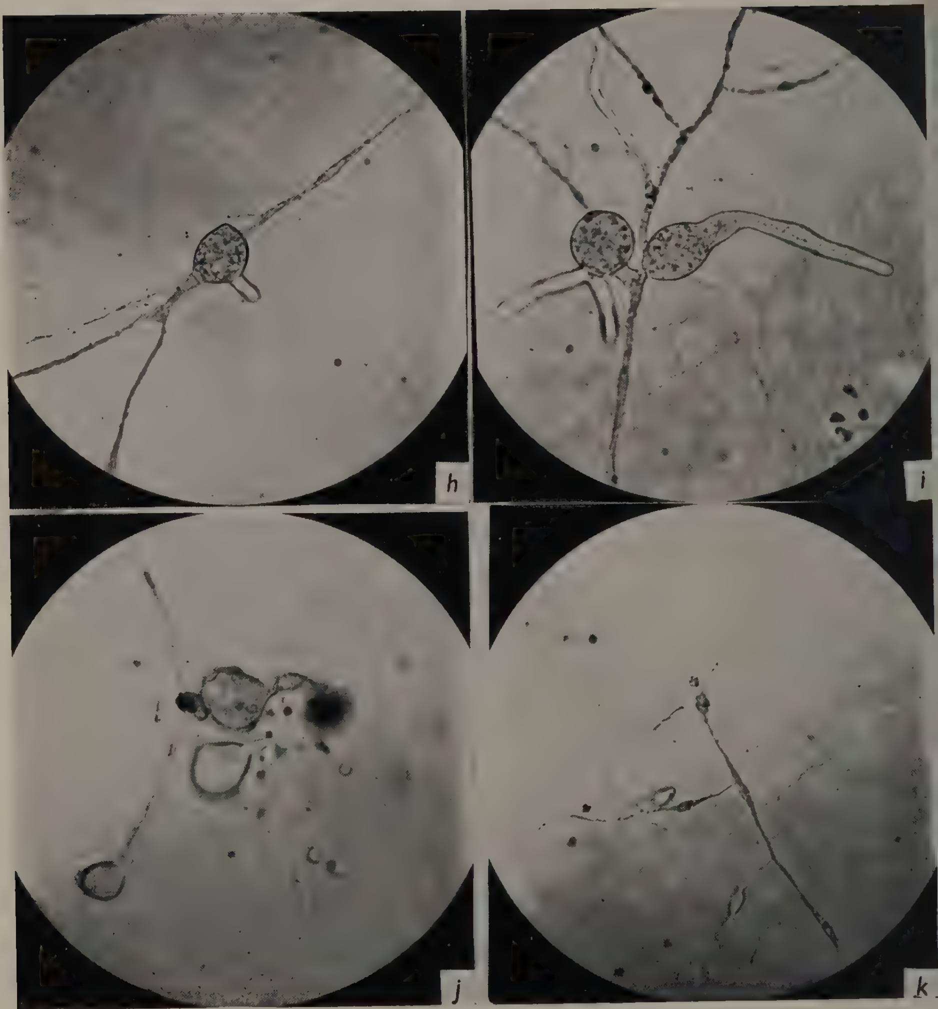
h
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Trans. Brit. mycol. Soc. **44** (3), 333–336 (1961).

RAMULARIA ARMORACIAE FUCKEL

By D. M. DRING

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(With 2 Text-figures)

A description is given of the development on horseradish of the lesions, conidia, stromata, spermogonia, and overwintering sclerotia which germinate in spring to give conidia.

In Devon and its adjoining counties horseradish (*Armoracia rusticana* Gaertn.) is commonly attacked by a fungus disease characterized by lesions, apparently only on the laminae, up to 1 cm. diam., usually of irregular shape and often coalescing. They appear as light brown, later grey or white, papery spots which may fall away leaving shot-holes. Small, dark coloured knots of mycelium are visible in the centre of the lesions but there is usually a broad (1–3 mm.) peripheral zone bearing none of these structures. The knots are hypophyllous at first but amphigenous later; zonation is not very marked.

The dark bodies may be tufts of conidiophores, or stromata, or spermogonia. A white powdering of conidia may sometimes be seen but is easily dispersed by rain, wind or careless collection. The conidia and conidiophores (Fig. 1*a*) are attributed to *Ramularia armoraciae* Fuckel (1870). Conidia from Devonian collections are occasionally septate, 6–14–24 × 3–4 μ, and conidiophores 25–50 × 3–4 μ.

The bunches of conidiophores become larger with age, and stromata consisting of dark brown pseudoparenchyma develop at their bases (Fig. 1 *b*). Hyaline tissue then develops within the stroma (Fig. 1 *c*) which eventually becomes a spermogonium. Often, the remains of conidiophores and even a few conidia are still attached to the mature spermogonium (Fig. 1 *d*), so that there can be no doubt as to the genetic relationship between these structures. Of 30 spermogonia selected at random, 22 had conidiophore bases (length 15–25–36 μ) still attached to them, making the whole structure typically cylindrical.

The spermogonia measure 30–45–70 μ diam. The spermatia (2·5–4·5 × 0·8–1 μ) are produced endogenously in flask-shaped mother cells (Fig. 1 *e*), whence they are discharged through a pore into the lumen of the spermogonium. The mass of spermatia and mucus escapes through the ostiole.

More or less spherical sclerotia, 25–45 μ diam., develop later embedded in the dead mesophyll of the lesions. They are composed of brown pseudoparenchyma with the largest and thinnest-walled cells on the inside and surrounded by a peridium one to two cells thick (Fig. 2*a*). Ordinary staining methods failed to reveal any protoplasmic contents in the sclerotium cells, and it may be supposed that protoplasm is limited to a thin layer lining each living cell.

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Material collected in the autumn and left out of doors until the following March contained germinating sclerotia. Germination begins by cells just inside the peridium becoming active, and developing strongly staining cytoplasmic contents. From these a group of hyaline conidiophores, which do not stain by any of the methods tried, grows out and ruptures the peridium. Easily stained conidia 10–25 × 3 μ are produced apically (Fig. 2*b*). Usually only one bunch of conidiophores is produced from each sclerotium but occasionally more were seen (Fig. 2*c*). With the development of the conidiophores disintegration of the sclerotium takes place,

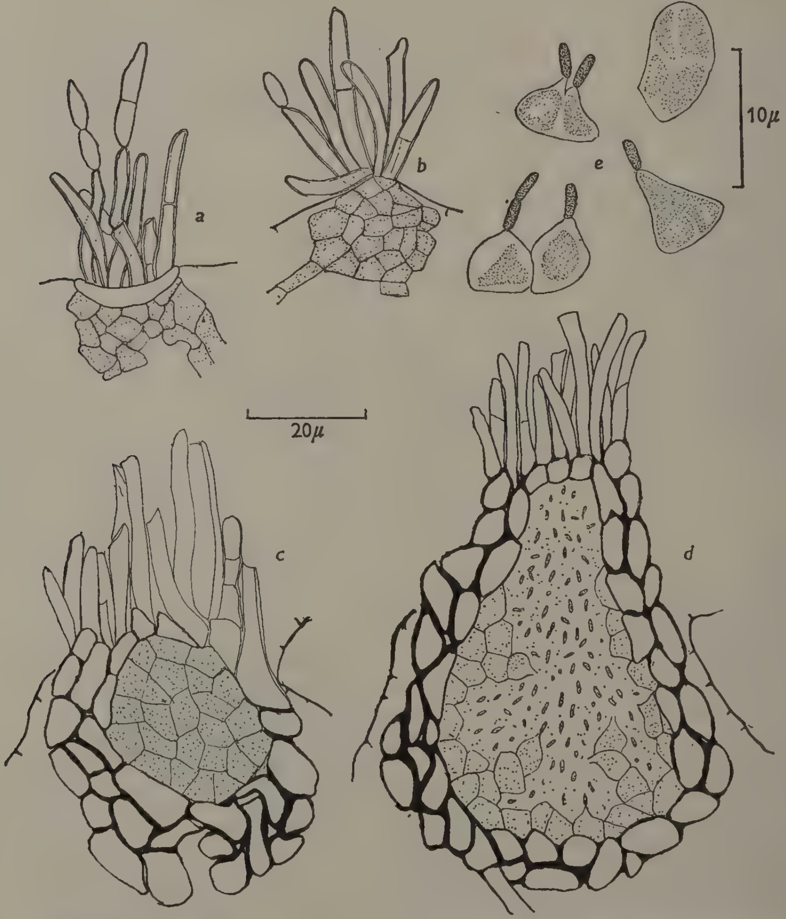

Fig. 1. *Ramularia armoraciae*. *a*, Tuft of young conidiophores; *b*, older group with developing stroma; *c*, developing spermogonium with conidiophores still attached; *d*, mature spermogonium; *e*, endogenous production of spermatia.

starting on the side away from the conidiophores and continuing until only the shrunken peridium is left (Fig. 2*d*).

Presumably, the germinating conidia infect the young horseradish leaves as they emerge in spring and the sclerotia are the means by which the fungus normally overwinters since, although a thorough search was made over three years of horseradish at every stage of development from several sites in Devon, no trace of a perfect state was found.

Cultures from the summer type of conidia were maintained for a considerable time on agar media. They bore only faint resemblance to the fungus *in vivo* and no organs other than conidia were produced. However, from their association on the same lesions and the germination of the sclerotium to produce *Ramularia*-like spores it seems entirely likely that all the organs described above are of *Ramularia armoraciae*.

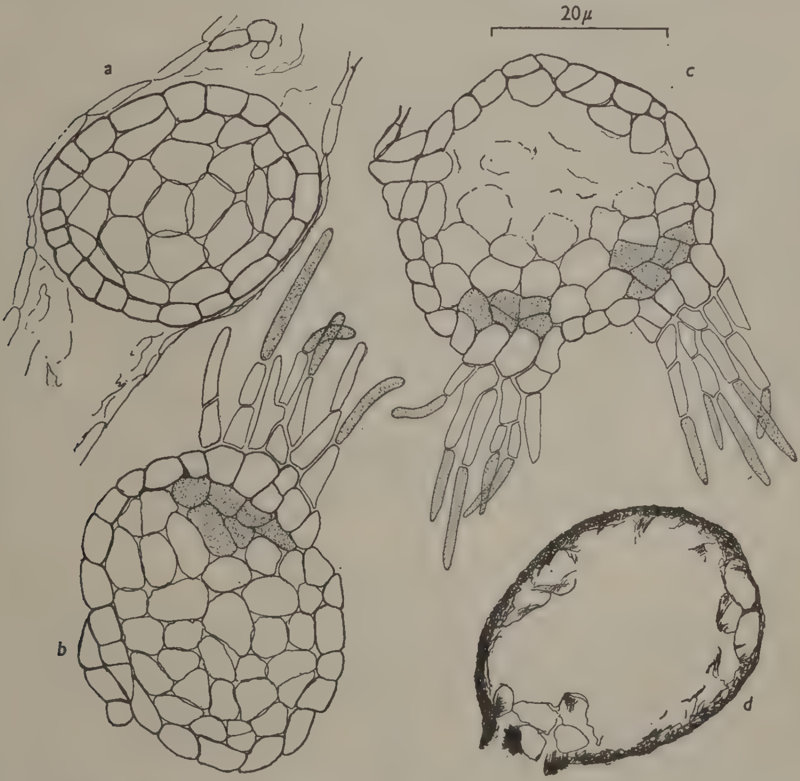

Fig. 2. *Ramularia armoraciae. a*, Sclerotium in dead mesophyll of horseradish; *b*, germinating sclerotium; *c*, sclerotium producing two tufts of conidiophores; *d*, exhausted sclerotium.

The similarity of the spermatia, and of their endogenous development, to those of *Mycosphaerella* spp. (Higgins, 1920, 1929, 1936; Dring, 1961) suggests that they are the imperfect state of a *Mycosphaerella*. Some *Mycosphaerella* spp. are known to have *Ramularia* states, e.g. *M. fragariae*

(Tul.) Lindau (*R. tulasnei* Sacc.). *R. vallisumbrosae* Cav. has a life-cycle which resembles that of *R. armoraciae*, particularly in the production of sclerotia which develop in a similar way to those described above. Gregory (1939) considered *R. vallisumbrosae* to be a fungus of the *Mycosphaerella* type whose perfect state has not yet been discovered.

Immature perithecia, probably of a *Mycosphaerella*, on horseradish have been described as *Sphaerella armoraciae* (Fuck.) Oudem. (Saccardo, 1882). No description of the mature perfect state has been found, and it may be assumed that if *R. armoraciae* is the imperfect state of a *Mycosphaerella* the perfect state is absent or very rare in south-west England, and over-wintering, normally a function of the perfect state in this type of fungus, is effected by the sclerotia.

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VENTURIA CARPOPHILA SP.NOV., THE ASCIGEROUS STATE OF THE APRICOT FRECKLE FUNGUS

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(With Plates 24 and 25)

The ascigerous state of the apricot freckle pathogen found on overwintering apricot leaves in Victoria, Australia, is described as *Venturia carpophila* sp.nov.; the pathogenicity of the fungus was established.

The disease commonly known as 'freckle' is to be found in most countries where apricots are grown. It is of considerable economic importance in parts of Australia (Fisher & Jenkins, 1954; Anon, 1953) and in South Africa it is particularly severe in summer rainfall areas (Bottomley, 1944). The pathogen has been known for many years in the conidial state, being first described as *Cladosporium carpophilum* Thümen (Saccardo, 1886; McAlpine, 1902) from peach fruits grown in Austria in 1877 and later transferred as *Fusicladium carpophilum* (Thüm.) Oud. Before the end of the nineteenth century the fungus was found in U.S.A. and Canada infecting plums and almonds as well as peaches (Smith, 1889; Pammel, 1892, 1893). In Australia the species was recorded on apricot fruits in 1910 (Anon, 1910; Noble *et al.* 1935). The ascigerous state, however, had not been discovered when the present investigation was undertaken.

ISOLATION OF THE FUNGUS

Towards the close of winter, August 1955, at Inverleigh, south-western Victoria, fallen leaves were collected from an apricot orchard where both leaves and fruits had been severely affected by 'freckle' in the preceding season. After being soaked thoroughly in water individual leaves were allowed to dry between two microscope slides according to the method described by Childs (1917). As the leaf tissue dried a number of bicellular, olive-buff (Ridgway, 1912, pl. 40) spores were ejected and deposited on the glycerin-coated surface of the contacting microscope slide (Pl. 24 fig. 1). These spores were $12–16 \times 3–5\,\mu$ and were divided by a median septum into two cells of equal length. One cell was rounded, sometimes almost spherical, and distinct from the adjacent, narrower, tapered cell.

Melted potato-dextrose agar, subsequently cooled to approx. 40° C., was poured in a thin film over the spores and the slides incubated for 24 hr. in sterile Petri plates at 25°. Agar pieces containing groups of two or three germinating spores were transferred with a sterile platinum needle to potato-dextrose agar slopes. Slowly growing, olivaceous black (3)

(Ridgway 51) colonies of characteristic cushion-like growth (cf. 'mammelon' described by Menon, 1956) were cultured in this way.

The tawny-olive (Ridgway 29), septate hyphae, 3–4 μ wide, gave rise to bluntly fusoid-obclavate, isabella coloured (Ridgway 30) conidia 12–18 × 4–5 μ, of a *Fusicladium* type.

PATHOGENICITY

Pathogenicity was examined in the spring, October 1955, on Moorpark apricot trees, growing in a 'freckle free' area at Burnley, Victoria. A sterile water suspension of conidia taken from a culture grown from two bicellular spores was applied by means of a fine atomizer to leaves and developing fruits of healthy apricot trees. Immediately after inoculation these branches were covered with polythene bags for 7 days. Control branches were treated in a similar manner with sterile water.

At the end of 5 weeks typical freckle symptoms appeared on the inoculated fruits; the lesions were 0·5–1 mm. diam., Acajou red (Ridgway 13) at first, but becoming mummy brown (Ridgway 15) and velvety, or freckle-like in appearance when mature (Pl. 24, fig. 5).

Leaf symptoms were not observed until 8 weeks after inoculation, when circular, snuff-brown (Ridgway 29) spots, approx. 0·5–1 mm. diam., appeared on the lower surface of the leaves. Also, linear lesions approx. 2 mm. long were found frequently on the veins and petioles (Pl. 24, fig. 5). The fungus was subsequently re-isolated from the infected leaves. Symptoms did not appear on control leaves or fruits.

TEMPERATURE RELATIONS

The effect of temperature on the viability of agar cultures was compared with that on cultures of *F. cerasi* (Rabenh.) Sacc. isolated from 'black spot' lesions on cherry fruits. Approx. 4 mm.^{2} of the culture to be tested was teased into fragments under aseptic conditions and suspended in 0·2 ml. water in a sterile Petri plate which was then poured with melted agar cooled to 40°. The medium used was Menon's malt extract agar (Menon, 1956), modified by replacing the apple and pear leaf decoction by one made from apricot or cherry leaves.

Experiment I

Two series of similar plates, inoculated, respectively, with *F. carpophilum* and *F. cerasi*, were set up in triplicate, incubated at 35–38° for 77 hr. and then transferred to 25° for 26 days. Apparently normal growth of the apricot pathogen occurred after this treatment, but the growth of *F. cerasi* was inhibited completely.

Experiment II

The growth of the two species was compared over a range of temperatures on potato-dextrose agar plus 0·1 % Marmite also conducted in triplicate, the inoculated plates being incubated at: (1) 35–39° for 73 hr., then transferred to 25° for 22 days; (2) −10° for 73 hr., then transferred to 25° for 22 days; (3) 25° for 25 days.

The resultant growth after 25 days indicated that a temperature of −10° did not inhibit the subsequent growth of either fungus at 25°, nor did this differ from normal growth at 25°. On the other hand, *F. cerasi* was much more sensitive than *F. carpophilum* to a temperature of 35–39° for 73 hr. Under the conditions of this experiment, 73 hours at 35–39° proved lethal to *F. cerasi*, but good growth of *F. carpophilum* occurred on transference to 25°.

HISTOLOGICAL EXAMINATION

Overwintered apricot leaves collected at Inverleigh in August 1955 were treated by the following techniques:

(i) Some leaves were cleared by soaking in a 1:1 mixture of chloral hydrate and carbolic acid crystals (Riker & Riker, 1936), then either mounted directly in lacto-phenol (Pl. 24, fig. 2) or teased into fragments and stained with cotton blue in lacto-phenol (Pl. 24, fig. 4).

(ii) Some leaves were fixed in formalin-acetic acid and embedded in paraffin wax. Microtome sections were cut at approx. 10 μ and stained with safranin and light green (Pl. 24, fig. 3).

Spherical to subspherical perithecia were found embedded in the leaf tissue and opening on to either the upper or lower surface by means of a well defined ostiole. These perithecia, which measured 60–160 μ diam. and were olive-brown (Ridgway 40), occasionally had bristles, but in general the perithecial wall may be described as glabrous (Pl. 24, fig. 2). At maturity the saccate-cylindrical asci, 48–56 × 5–6 μ, contained 8 bicellular olive-buff (Ridgway 40) ascospores (Pl. 24, figs. 3, 4). The upper cell of the ascospore, i.e., the cell directed towards the apex of the ascus, was invariably broader than the lower cell, which was usually slightly tapered.

These ascospores may be identified, on the basis of colour, size and shape, with the bicellular spores ejected from water-soaked over-wintering apricot leaves (Pl. 24, fig. 1).

IDENTIFICATION

The evidence presented here indicates that the apricot freckle pathogen can overwinter in an ascigerous condition, developing on fallen infected apricot leaves.

Venturia carpophila sp.nov. (Pl. 24, figs. 1–4).

Hyphae septatae, fulvo-olivaceae, 3–4 μ diam.; perithecia in substantia folii immersa sed ad maturitatem erumpentia, globosa vel subglobosa, ostiolata, usitate glabra (setae solum interdum adsunt), olivaceo-brunnea, 60–160 μ diam.; asci saccato-cylindracei, 8-spori, 48–56 × 5–6 μ; ascosporae 1-septatae, cellulis aequilongis (cellula inferiore contracta, atque cellula superiore latiore), olivaceo-ochroleucae, 12–16 × 3–5 μ.

Status asexualis *Fusicladium carpophilum* (Thüm.) Oudem. syn. *Cladosporium carpophilum* Thüm. *Symb. Mycol.* p. 107 (1887) est, conidia isabellina, obtuse fusiformia vel obclavata, 12–18 × 4–5 μ.

Habitatio, in foliis mortuis Pruni armeniacae. Typus, Inverleigh, Victoria, Australia; leg. E. E. Fisher, 29 Aug. 1955 (in Herb. Dep. Agric. Vict.; isotypus in K).

Hyphae septate, tawny-olive (Ridgway 29), 3–4 μ diam.; perithecia embedded in leaf tissue, but erumpent at maturity, spherical-subspherical, ostiolate, generally glabrous, only occasionally with bristles, olive brown (Ridgway 40), 60–160 μ diam.; asci saccate-cylindrical, 8-spored, 48–56 × 5–6 μ; ascospores divided by a median septum into 2 cells of equal length, upper cell wider than lower tapered cell, olive-buff (Ridgway 40), 12–16 × 3–5 μ.

Conidial state *Fusicladium carpophilum* (Thüm.) Oudem. syn. *Cladosporium carpophilum* Thüm. *Symb. Mycol.* p.107 (1887). Conidia, bluntly fusoid-obclavate, isabella (Ridgway 30), 12–18 × 4–5 μ.

Habitat, on dead leaves of *Prunus armeniaca*, Inverleigh, Victoria, Australia, E. E. Fisher, 29 August 1955. Type in Victorian Department of Agriculture, Australia; cotype in Herb. Kew, England.

Discussion

In the pathogenic state the fungi responsible for apricot freckle and cherry scab, are conidial forms which have been referred to *F. carpophilum* (Thüm.) Oudem. and *F. cerasi* (Rabenh.) Sacc., respectively.

From an early date an ascigerous state also was known to occur in the life cycle of the cherry scab pathogen, which was described by Saccardo (1878, 1882) as *V. chlorospora* (Ces.) Karst. var. *pruni-cerasi*. Later Aderhold (1900) gave a detailed description of this fungus and referred it to a new species, *V. cerasi*. Menon (1956) re-named the cherry fungus *V. pruni-cerasi* (Sacc.) Menon, but this is antedated by *V. cerasi*.

When the present investigation was undertaken there was no evidence of an ascigerous state in the life cycle of *F. carpophilum*. Comparative studies of freckle and scab diseases of stone fruits have been made by several investigators. Bensaude & Keitt (1928) concluded that the cherry isolate was quite distinct from *F. carpophilum*. They also formed the opinion that the 'genetic and taxonomic relationships...of *Cladosporium* spp. of stone fruits...are likely to be somewhat doubtful until more is known of their possible ascigerous stages'.

Schweizer (1958) made comparative biological studies of the cherry and peach scab pathogens and also concluded that they must be regarded as two separate species. He studied the annual cycle of *F. cerasi* in detail and found in old cherry leaves perithecia and ascospores,which conformed to the description of *V. cerasi* given by Aderhold (1900). With peach scab, on the other hand, Schweizer was able to find only the conidial state (*F. carpophilum*), and overwintering appeared to be by infected shoots of the host plant.

When, in the present study, an ascigerous state was found to develop in fallen apricot leaves from 'freckle' infected trees, a comparison with *V. cerasi* was considered to be desirable. As no authentic herbarium material of *V. cerasi* was available, this comparison has been made by reference to the original description and the accompanying illustration given by Aderhold (1900).

It would seem that the apricot and cherry pathogens differ quite consistently in ascospore morphology. The position of the septum and relative size of the two cells of the ascospore have been used by Menon (1956) and others to differentiate between species of *Venturia*. In *V. cerasi* the upper cell of the ascospore is characteristically pointed and it is longer than the lower. The apricot pathogen, on the other hand, is characterized by ascospores divided by median septa into two cells of equal length; the upper, which is conspicuously broader than the lower, is rounded, sometimes almost spherical, but never pointed as in *V. cerasi*.

It has been demonstrated during the course of the present investigation that, in addition to these morphological differences, the apricot freckle fungus is more resistant than *V. cerasi* to high temperature conditions.

These results tend to confirm the suggestion made by Bensaude & Keitt (1928) that 'the *Cladosporium* which occurs on *Prunus cerasus* has a somewhat lower thermal range for germination and growth than have the strains which parasitize *P. americana* and *P. persica*'. Furthermore they, and more recently Schweizer (1958), have demonstrated that these fungi exhibit differences in pathogenicity. *F. cerasi* is of limited host range and appears to be almost completely specific to cherry, whereas *F. carpophilum* has been found on a variety of stone fruits, including peaches, plums and apricots.

All these facts support the view that the apricot freckle fungus is a distinct species.

I am indebted to Mr J. H. Willis, B.Sc., National Herbarium of Victoria, for the Latin diagnosis; and I wish to thank him for his very willing help in this matter.

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EXPLANATION OF PLATES 24 AND 25

PLATE 24

Venturia carpophila

Fig. 1. Ascospores ejected from dead apricot leaf. × 400.

Fig. 2. Perithecia in dead apricot leaf after clearing in carbolic acid + chloral hydrate. × 100.

Fig. 3. Microtome section of perithecium stained with safranin and light green. × 400.

Fig. 4. Asci containing ascospores emerging from ruptured perithecium, after clearing in carbolic acid + chloral hydrate. Stained with cotton blue. × 480.

Figs. 5, 6. Demonstrating the pathogenicity of cultures (*Fusicladium carpophilum*) grown from ascospores of *V. carpophila*.

Fig. 5. Apricot leaves 10 weeks after inoculation.

Fig. 6. Above: control apricot fruits. Below: fruits 8 weeks after inoculation.

PLATE 25

Illustrating the effect of temperature on cultures of: A, *Fusicladium carpophilum* (grown from ascospores of *Venturia carpophila*); and B, *Fusicladium cerasi* (isolated from cherry fruits).

Fig. 7. Petri plates incubated at 35–38° for 77 hr. then 25° for 26 days.

Fig. 8. Petri plates incubated at (left–right): (1) 35–39° for 73 hr. then 25° for 22 days; (2) −10° for 73 hr. then 25° for 22 days; (3) 25° for 25 days.

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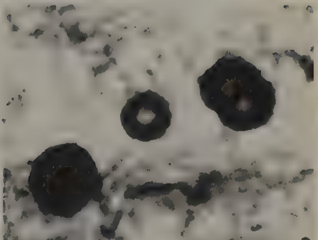

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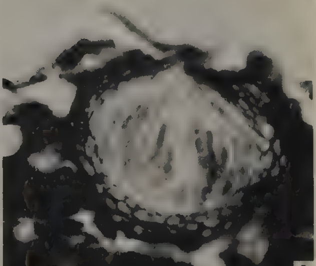

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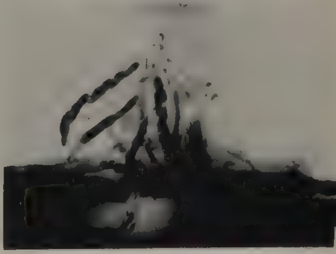

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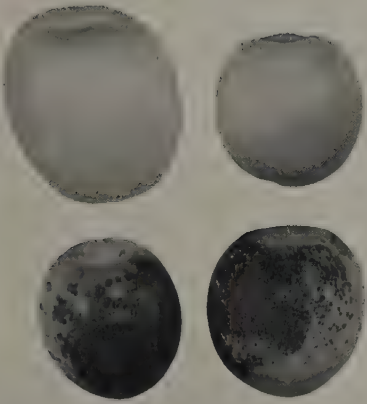

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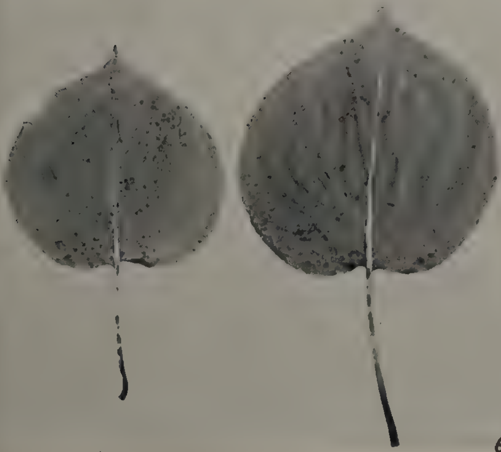

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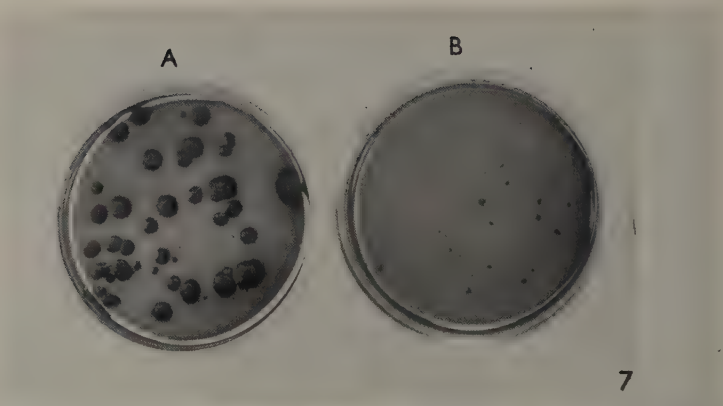

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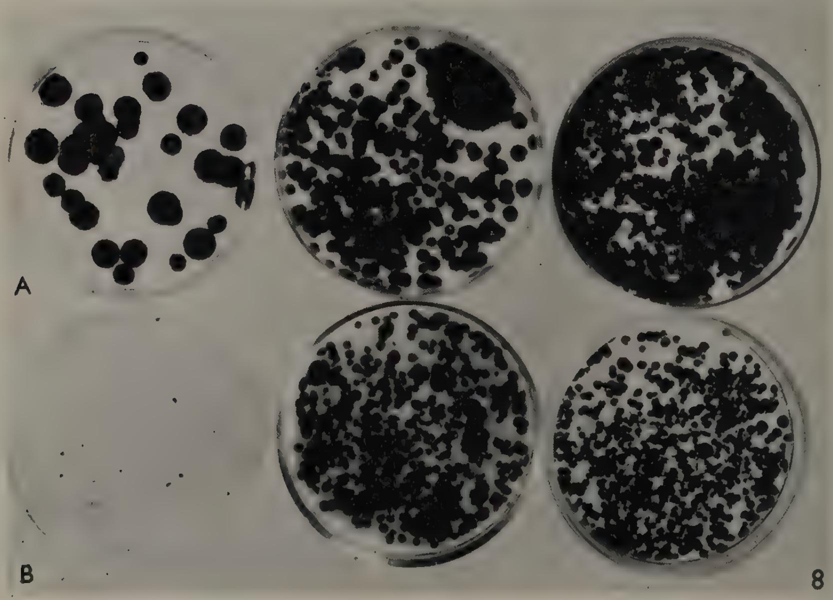

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SOME FACTORS AFFECTING OCCURRENCE OF *FUSARIUM CULMORUM* IN THE SOIL

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(With 6 Text-figures)

During a survey of soil fungi in plots of perennial rye-grass, *Fusarium culmorum* (W. G. Sm.) Sacc. was regularly isolated over a period of 21 months. Frequency of isolation was significantly greater from plots which had been limed and from those receiving nitrogen; there was, however, no evidence of an interaction between lime and nitrogen. There was also a significant seasonal variation in frequency of isolation. This could not be related simply to either soil moisture or temperature considered independently, but there was evidence of interaction in that low frequencies were associated with either (*a*) low temperature ($< 7^\circ$ C.) and high moisture or (*b*) high temperature ($> 7^\circ$) and low moisture; higher frequencies, on the other hand, were associated with high temperature coupled with high moisture. Frequency of recovery of the fungus from soil samples incubated in the laboratory for 28 days, under a wide range of temperature and moisture conditions, followed closely that found in the field.

The inhibitory effect of high temperature at low moisture levels was not observed in sterilized soil, and it has been concluded that under these conditions microbial antagonism plays a significant role in determining the activity of *F. culmorum*. It has been found that, in sterilized soil at low moisture levels, *Trichoderma viride* suppresses growth of *F. culmorum* at temperatures above 10°, whereas at lower temperatures *F. culmorum* suppresses *T. viride*.

Early studies on *Fusarium culmorum* (W. G. Sm.) Sacc. were mainly concerned with its activities as a pathogen causing a brown root rot of cereals. More recently it has been shown that it is also an active saprophyte. Wheat straws buried in soil are readily colonized (Sadasivan, 1939; Walker, 1941; Butler, 1953; Lucas, 1955) as also are senescent roots of wheat (Broadfoot, 1933; Samuel & Greaney, 1937; White, 1945), oats (Parkinson & Chesters, 1958) and rye-grass (Waid, 1957). The fungus is apparently regularly present in cultivated soils (Sadasivan, 1939) and has also been found in abundance in soils which are rarely if ever cultivated (Stenton, 1953).

While much is known about the distribution of the fungus, less attention has been paid to the influence of environmental factors on its relative abundance in the soil. In order to provide further information on this aspect, use has been made of data collected during a survey of soil fungi in plots of perennial rye-grass. *F. culmorum* was regularly isolated during a period of 21 months, and an attempt has been made to relate variation in its seasonal occurrence to environmental factors.

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Materials and methods

Experimental plots. Thirty-two small plots (5 ft. × 8 ft.) of S24 perennial rye-grass were established in the Department's Experimental Gardens in the spring of 1954. The soil type in the area occupied by the plots belongs to the Sannon Series and is a deep colluvial soil with imperfect drainage at a depth of 12–18 in. The texture of the fine earth material is rather heavy, but the high gravel and stone content and a good crumb structure confer the working properties of a lighter soil.

The plots (arranged in two quasi-Latin squares) were subjected to a variety of manurial and cutting treatments as shown below:

G	NL	GNL	—
GNL	—	G	NL
L	GN	N	GL
N	GL	L	GN

Latin square A

G	NL	L	GN
GNL	—	N	GL
L	GN	GNL	—
N	GL	G	NL

Latin square B

G: plots cut each month from April to September inclusive each year. Other plots cut once only, generally toward the end of June each year.

N: plots receiving nitrogen. Applied as sulphate of ammonia: 2 cwt./acre in April, June and August of each year.

L: plots receiving lime. Applied as calcium hydroxide: 3 tons/acre prior to sowing in 1954 and 1 ton/acre in the springs of 1956 and 1957.

Table 1. *Summary of samples taken and plates examined during survey January* 1956–*September* 1957

Period	Months	Latin square	No. plots sampled per month	No. soil plates per plot
I	Jan., May, Mar.	A	16	3
		B	—	—
II	July, Sept., Nov.	*	8	20
III	Jan., Mar., May, July, Sept.	A	—	—
		—	16	10
	Feb., Apr., June, Aug.	B	—	—

* All plots receiving lime and nitrogen as single treatments.

A meteorological station was not available on the site of the experimental plots and use was therefore made of data collected at the Welsh Plant Breeding Station's farm (Frongoch) nearby.

Collection of soil samples and isolation of fungi. Soil samples from each plot comprised three cores (1 in. × 5 in.); these were broken up, and the soil was thoroughly mixed and passed through a 2 mm. sieve. The frequency of sampling and number of samples examined was not constant throughout the survey. Since this has necessarily influenced the reliability of estimates for *F. culmorum*, a summary of samples taken and number of plates examined is given in Table 1.

The fungi were determined from soil plates (Warcup, 1950). In each plate approx. 5 mg. soil was incorporated in 10 ml. agar medium of the following composition: glucose, 10 g.; potassium nitrate, 1 g.; potassium dihydrogen phosphate, 1 g.; agar, 20 g.; rose bengal, 67 p.p.m.; tap water, 1 l. The reaction of the medium was adjusted to pH 6. Plates were incubated, except where stated otherwise, at 22° and kept under observation for 28 days. It was not practicable to count the number of colonies of each species which appeared on the plates. Consequently the estimates of *F. culmorum* are based on the proportion of plates on which it occurred.

Measurement of growth in sterile soil. In order to measure the growth of fungi (either singly or in combination) in sterile soil, special growth tubes were used. These were essentially similar to those described by Evans (1954) and consisted of 14 in. lengths of ½ in. diameter glass tube with six side arms at intervals of 1½ in. The tubes were filled with sieved soil and, after plugging ends and side arms with cotton-wool, were autoclaved for 30 min. at 1 atm.

Difficulty was encountered in adjusting the moisture content of soil in the tubes. If water was added through the side arms after filling, the moisture content showed marked variation along the tube. Adjustment of water content prior to filling was satisfactory up to a level of 40% M.H.C., but above this the crumb structure broke down during filling. Because of these difficulties the moisture levels studied were restricted to 25, 40 and 100% M.H.C., the last being obtained by saturating a filled tube and allowing it to drain for 24 hr.

OBSERVATIONS AND EXPERIMENTAL RESULTS

Effect of nitrogen, lime and cutting on the occurrence of Fusarium culmorum

Estimates of *F. culmorum* for 1956 were unsuitable for detailed analysis of the effects of treatments; in period I, estimates were based on a relatively small number of samples, and in period II only plots receiving nitrogen and lime as single treatments were studied. However, it can be seen from Tables 2 and 3 that nitrogen consistently tended to favour the occurrence of the fungus.

Table 2. *Occurrence of* Fusarium culmorum *during period I*

Month* (1956)	Treatments N	−N	L	−L	G	−G	Total
Jan.	15	11	15	11	11	15	26
Mar.	3	1	3	1	3	1	4
May	6	3	3	6	1	8	9

* In any month the maximum possible frequency in any treatment is 24.

Table 3. *Occurrence of* Fusarium culmorum *during period II*

Month* (1956)	Treatment N	L	Total
July	6	1	7
Sept.	27	16	43
Nov.	43	40	83
Total	76	57	133

* In any month the maximum possible frequency in either treatment is 80.

In period III (January–September 1957) estimates were based on a much larger number of samples and all combinations of treatments were included. An analysis of these data is shown in Table 4. It can be seen that lime, in addition to nitrogen, produced highly significant increases in the abundance of the fungus; moreover, the effects of these two treatments would appear to be strictly additive since the nitrogen–lime interaction (N/L) was extremely small. The effects of lime and nitrogen are shown graphically in Figs. 1 and 2, respectively. No significant effects attributable to cutting alone could be detected but there was an indication of an interaction (significant at the 5 % level) with time of sampling.

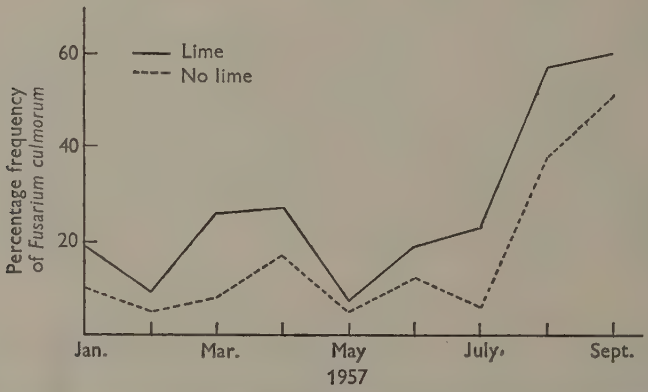

Fig. 1. Effect of lime on the occurrence of *F. culmorum* in soil from plots of perennial rye-grass.

Table 4. *Effect of treatments and time of sampling on the occurrence of* Fusarium culmorum *during period III*

Analysis of variance

Item	S.S.	D.F.	M.S.	V.R.
Latin squares	372	1	372	2·3106
Rows	1,186	6	198	1·2298
Columns	2,330	6	388	2·4100
Nitrogen (N)	2,704	1	2,704	16·7950**
Cutting (C)	121	1	121	0·7516
Lime (L)	5,329	1	5,329	33·0994***
N/C	171	1	171	1·0621
L/C	82	1	82	0·5093
N/L	11	1	11	0·0683
Squares/N	753	1	753	4·6770
Squares/C	78	1	78	0·4845
Squares/L	110	1	110	0·6832
Error (A)	1,453	9	161	—
Months (M)	35,576	7	5,082	16·6623***
M/N	3,104	7	443	1·4525
M/C	5,524	7	789	2·5869*
M/L	1,074	7	153	0·5016
M/Rows	12,728	21	606	1·9869
M/Columns	5,302	21	252	0·8262
M/NC	2,190	4	548	1·7967
M/LC	2,085	4	521	1·7082
M/NL	2,537	3	846	2·7738
Error (B)	9,462	31	305	—

From Fig. 3 it can be seen that, during the spring and summer, the plots cut monthly gave values greater than or equal to those cut once. In the autumn and winter this situation was reversed.

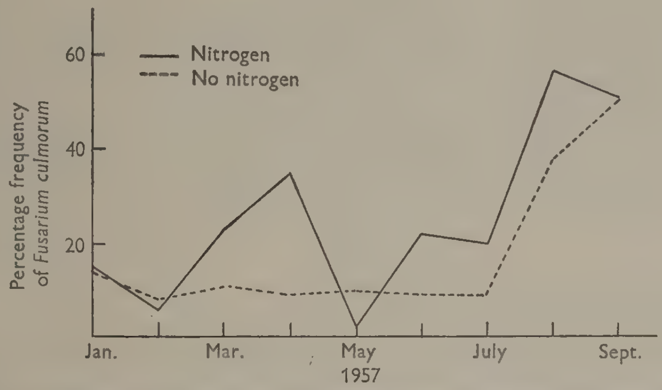

Fig. 2. Effect of nitrogen on the occurrence of *F. culmorum* in soil from plots of perennial rye-grass.

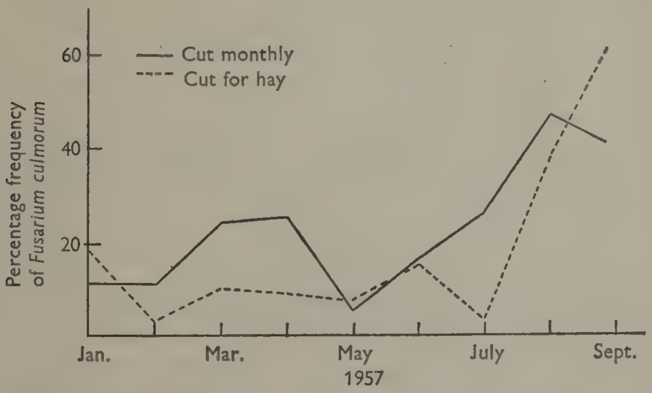

Fig. 3. Effect of different systems of grass cutting on the occurrence of *F. culmorum* in soil from plots of perennial rye-grass.

Seasonal variation in occurrence of Fusarium culmorum

Although the treatments applied to the experimental plots produced significant differences in the abundance of *F. culmorum*, these effects were small in comparison with differences associated with time of sampling, i.e. seasonal fluctuations. Consequently the data for all plots was, for each sampling occasion, combined to provide a single value. These combined values were expressed as percentages, to make comparable the estimates made during the three periods of the survey. It can be seen (Fig. 4) that in both 1956 and 1957 the fungus was generally most abundant during the autumn months (September–November) and in late spring (April–May).

In attempting to relate these seasonal differences to temperature and moisture, comparison was made between estimates of *F. culmorum* and the conditions prevailing in the month prior to sampling. Comparison with temperature presented no difficulties, since detailed records of soil temperature at 4 in. depth were available. No records, however, were available for soil moisture. Nevertheless, using Penman's (1954) formula it was possible to obtain, from the meteorological data, some idea of the soil moisture status or, more precisely, the extent of the moisture deficit during the summer months.

Neither soil temperature nor moisture considered independently showed any relationship with the recorded frequencies of *F. culmorum*. The

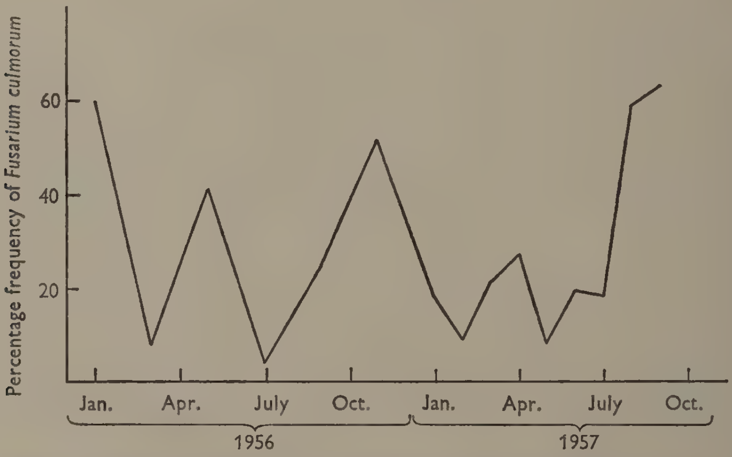

Fig. 4. Seasonal distribution of *F. culmorum*, January 1956–September 1957.

Table 5. *Occurrence of* Fusarium culmorum *in relation to soil temperature and moisture*

Sampling date	Mean soil temp. (°C.) in previous month	Soil moisture deficit (in.) at end of previous month	Percentage frequency of *F. culmorum*
	Group 1: low frequencies		
July 1956	16·5	3·40	4
Mar. 1956	1·2	0	8
May 1957	8·8	1·29	8
Feb. 1957	4·5	0	9
Jan. 1957	6·2	0	18
July 1957	15·3	4·70	18
June 1957	11·4	2·79	19
Mar. 1957	4·5	0	21
Sept. 1956	13·3	0	25
Apr. 1957	7·0	0	28
	Group 2: high frequencies		
May 1956	6·6	1·11	41
Nov. 1956	10·8	0	51
Aug. 1957	14·0	0	59
Jan. 1956	6·0	0	60
Sept. 1957	15·8	0	64

possibility of an interaction between these factors was considered in the following way. The recorded frequencies of *F. culmorum* ranged from 4 to 66% and these values were arbitrarily divided in Table 5 into two groups: group 1, low frequencies, < 40%; group 2, high frequencies, > 40%.

From Table 5 it can be seen that, in general, there is a tendency for low frequencies to be associated with either (*a*) low temperature (< 7°) and high moisture (no moisture deficit), or (*b*) high temperature (> 7°) and low moisture (moisture deficit > 1 in.). The higher values, on the other hand, appear to be related to high temperature coupled with high moisture. The few departures from this general trend occurred in 1956 when, because of the smaller number of samples analysed, less significance can be attached to the estimates of *F. culmorum*.

Recovery of Fusarium culmorum *from soils incubated under controlled conditions*

The effect, on the soil fungi, of incubating soil under known conditions of temperature and moisture was also investigated. Soil, comprising cores from all plots, was thoroughly mixed and air-dried in shallow trays for 7 days at room temperature. Such soil was regarded, for the purposes of this experiment, as having a zero moisture content. The M.H.C. was determined, and samples having 25, 50, 75 and 100% M.H.C. were then prepared. These were distributed to conical flasks plugged with cotton-wool; two flasks for each moisture level were incubated for 28 days at each of the following temperatures: 5, 10, 15, 20 and 25°. At the end of the 28-day period, soil plates were prepared in the usual way. Of the plates prepared only four (two from each flask) were incubated at the standard temperature of 22°. Thus, for any one species of fungus, it might be expected that only gross differences would be detected. However, the data for *F. culmorum* showed a number of interesting features (Fig. 5). The pattern of recovery of *F. culmorum* from these soils incubated under different conditions followed closely that found in soils taken from the field at different times. The condition of cold, dry soil, which under laboratory conditions favoured *F. culmorum*, was not recorded in the field in either 1956 or 1957. However, both sets of results showed low frequencies to be associated with both cold, wet soil and warm, dry soil; and high frequencies with warm wet soil.

Influence of Trichoderma viride *on* Fusarium culmorum

In unsterilized soil, moisture and temperature show a marked interaction in their influence on the occurrence of *F. culmorum*. This is not so in sterilized soil, growth rates in which were determined at three moisture levels (25, 40 and 100% M.H.C.) and at five temperatures (5, 10, 15, 20 and 25°). Growth rate was not significantly affected by differences in moisture status, but increased almost linearly with temperature over the range studied (Fig. 6).

This difference in behaviour in sterilized and unsterilized soil is not surprising and suggests that the general microbial population is exerting some effect. It has not been possible to examine this in detail, but the results obtained from a study of the influence of one species, *Trichoderma viride*, indicate how the observed differences may come about. *T. viride* was chosen for study because of its well known antagonistic properties, its abundance in the soil under investigation and because it may occupy substrates similar to those of *F. culmorum*.

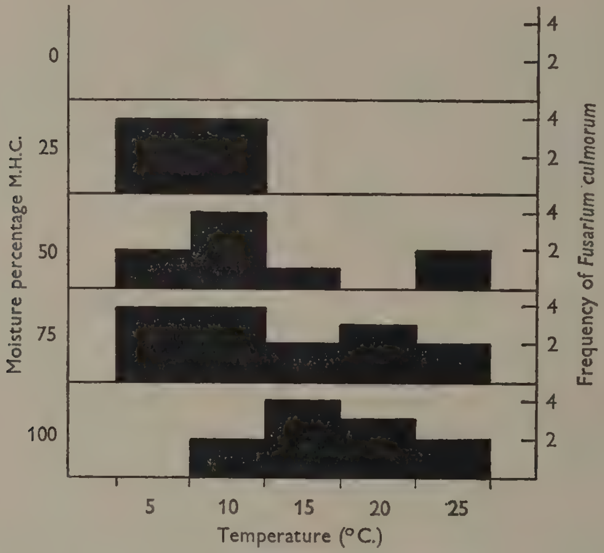

Fig. 5. Recovery of *F. culmorum* from soils incubated at various temperatures (5–25°) and moisture levels (0–100 % M.H.C.).

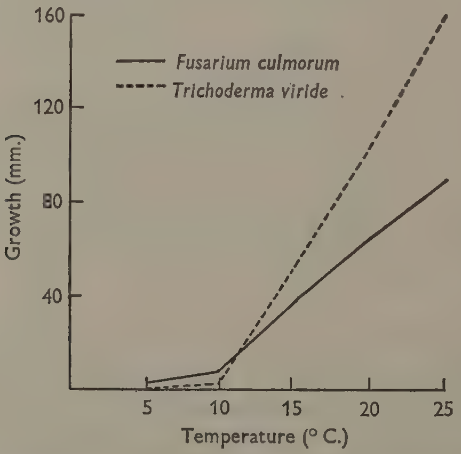

Fig. 6. Effect of temperature on growth of *F. culmorum* and *Trichoderma viride* in sterilized soil.

In sterile soil *T. viride* grew well at all three moisture levels but differed from *F. culmorum* in its response to temperature. Whereas *F. culmorum* made significant growth at 5°, *T. viride* made no growth at this temperature.

In soil tubes co-inoculated with both species the following results were obtained. At 5° and 10°, *F. culmorum* was the only species recovered, while at the higher temperatures the position was reversed and only *T. viride* was recovered. This result was obtained at all three moisture levels studied.

DISCUSSION

The problem of assessing fungal activity in the soil has recently been reviewed by Warcup (1960) and it is evident that there is a tpresent no simple means whereby a distinction may be made between active mycelium and spores. Quantitative estimates of a fungus derived from dilution plates (and the fundamentally similar soil plates of Warcup, 1950) are essentially estimates of spores (Warcup, 1955). For this reason these methods have been considered of little value for quantitative studies on fungal activity. However, in the absence of more satisfactory techniques of equal simplicity, we may well wonder whether this is too hasty a judgement. It is clear that a single quantitative estimate from dilution plates is of very restricted value, but the same is not necessarily true of a succession of estimates made at relatively frequent intervals for here it is possible to detect changes in the population of spores. At any one time the number of viable spores of a species will be the resultant of two processes: (*a*) sporulation leading to an increase, (*b*) spore destruction and inviability leading to a decrease. It must be conceded that an increase in spore number reflects the occurrence, either contemporaneously or in the past, of mycelial activity: equally for many fungi a decline in spore number will reflect a reduction or cessation of mycelial growth. Thus the spore population may be regarded as a barometer of fungal activity and, just as with an ordinary barometer, it is change in value rather than absolute value which is of interest.

In the case of *F. culmorum*, the sharp declines in frequency which occur over short periods provide grounds for believing that the spore population is not highly persistent. Equally, although there is no direct evidence about the growth and reproduction of *F. culmorum* in soil, its behaviour both in culture and on plant material suggests that spore production may follow rapidly upon mycelial growth. For this species then, it appears that estimates of the spore population may provide a useful guide to its relative activity in the soil.

Previous studies on the occurrence of *F. culmorum* in the soil have emphasized its association with freshly incorporated plant residues. This is particularly evident in wheat fields where high incidence of *F. culmorum* regularly occurs at the end of the season, when substantial quantities of root material and stubble become available for colonization (Samuel & Greaney, 1937; Warcup, 1957). Under grassland conditions, however, there appears to be little evidence to suggest that the occurrence of *F. culmorum* is substantially influenced by the annual cycle of root production and decay. In rye-grass, as in many other grasses, new root production occurs mainly in the spring and precedes the main flush of top growth which culminates in flowering in early summer. Senescence and death of the previous season's roots tend to follow closely the production of new roots (Troughton, 1957). In general then, fresh substrate would tend to become available about April. Peaks of activity were recorded for *F. culmorum* in May 1956 and April 1957, and it may well be that increase in available substrate at this time may have contributed to the increased activity. Unfortunately this period regularly coincides with increasing soil temperature, and it is consequently not possible to dissociate the effect of temperature from the effect of increased substrate. In view of the generally good agreement between field results and laboratory data for soils incubated under controlled conditions it would seem, however, that under grassland conditions seasonal variation is primarily attributable to differences in moisture and temperature.

The response of *F. culmorum* to temperature in dry soils both in the field and in the laboratory has provided a striking example of the importance of this factor in determining the outcome of competitive relationships between micro-organisms. Several workers (Henry, 1932; Schroeder & Walker, 1942) have shown that root diseases in unsterilized soil have a lower optimum temperature for development than the optimum for the growth of the parasite. In sterilized soil, however, the optimum temperatures for the development of the disease and for the growth of the parasite, respectively, tend to be the same. These observations have led to the view that in these cases antagonism is greater at higher temperatures. Fellows (1941) drew similar conclusions from a study of the survival of *Ophiobolus graminis*; he found that survival was less in warm as opposed to cool soils. More recently, Rishbeth (1950) has investigated competition between *Fomes annosus* and various soil saprophytes in roots of pine. Again it was found that, as temperature increased, competition by *F. annosus* was less effective.

The present study has shown that in sterile soil temperature markedly affects antagonism towards *F. culmorum* by *Trichoderma viride*. At temperatures below 10° *F. culmorum* is wholly dominant, whereas at higher temperatures the situation is reversed. This behaviour so closely parallels that found in the field and in the laboratory under dry soil conditions that it is tempting to suggest that *T. viride* is the actual antagonist or competitor involved. Moreover, *T. viride* was abundant in the soil studied and may well tend to occupy the same substrates as *F. culmorum*, for it has regularly been observed fruiting on pieces of organized plant remains on the soil.

These results may provide a plausible explanation for the reduced activity of *F. culmorum* at high temperature in soils of low moisture content; they do not, however, account for the high activity when the soil is wet, since in sterile soil *T. viride* suppressed *F. culmorum* at all moisture levels. It appears possible that in non-sterile soil the activity of *T. viride* is reduced at high moisture levels and there is some evidence to suggest that this is so. In soils at moisture contents of 75 and 100 % M.H.C. incubated at 20° and 25°, *T. viride* was observed to be much less abundant on soil plates and was occasionally absent. At lower moisture levels, however, it was regularly abundant (Siddiqi, 1958). The reasons for this apparent reduction in the activity of *T. viride* under these conditions are at present unknown.

We are indebted to the Welsh Plant Breeding Station for making available the meteorological data, to Dr Alan Durrant of the Department of Agricultural Botany, U.C.W., Aberystwyth, for advice on the statistical treatment of the data and to Dr W. L. Gordon, Dominion Rust Research Laboratory, Winnipeg, for confirming the identity of our cultures of *Fusarium culmorum*.

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FLAVIPIN PRODUCTION BY *EPICOCCUM* SPP.

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An antibiotic produced by *Epicoccum nigrum* Link and *E. andropogonis* (Ces.) Schol-Schwarz has been isolated and shown to be flavipin (3,4,5-trihydroxy-6-methylphthalaldehyde), a known metabolite of *Aspergillus flavipes* and *A. terreus*. It is an antifungal substance, only stable in aqueous solution at low pH.

Campbell (1956) has shown that when *Epicoccum purpurascens* and *Helminthosporium sativum* were grown side by side on agar media, the former fungus produced a diffusible substance which restricted hyphal growth of *H. sativum*. We have confirmed the production of an antifungal antibiotic by *E. nigrum* (= *E. purpurascens*) and by *E. andropogonis*. This has been isolated and shown to be identical with flavipin (3,4,5-trihydroxy-6-methylphthalaldehyde) previously obtained from *Aspergillus flavipes* and *A. terreus* by Raistrick & Rudman (1956).

MATERIALS AND METHODS

Methods of surface culture and assay of antibiotic activity have been described previously (Curtis, Hemming & Unwin, 1951). Stirred cultures were grown in conventional glass and stainless steel fermenters containing 6 l. medium, stirred at 700 r.p.m. and with an air flow of 3 l./min. All cultures were grown at 25° C. The media used were Czapek-Dox and Raulin-Thom (Brian, Curtis & Hemming, 1946) and medium T, containing Bacto-Tryptone as nitrogen source (Brian, Curtis & Hemming, 1947). Difco yeast extract (0·01 %) was added to all media. Pea-stem section extension and cress-root growth tests have also been described elsewhere (Brian & Hemming, 1958; Brian, Radley & Hemming, 1955).

The fungi used included three received from the Commonwealth Mycological Institute, Kew, as *E. purpurascens* (CMI. 45171, 61336 and 68797), four received from the Centraalbureau voor Schimmelcultures, Baarn, as *E. nigrum* ('Portugal C', 'Mulder', 724 and '*E. neglectum*') and four more, from the same source, as *E. andropogonis*.

RESULTS

Preliminary experiments

Cultures were grown on 30 ml. lots of Czapek-Dox and Raulin-Thom media in 100 ml. conical flasks and assayed for antifungal activity at intervals for a total of 28 days. All grew vigorously on both media producing dark-red, reddish brown or orange-brown culture filtrates. The filtrates were noticeably viscous in most cases. They were in all cases antifungal, particularly on Raulin-Thom, the most active filtrates being produced by *E. purpurascens* (CMI, 68797). On Raulin-Thom medium this strain produced filtrates inhibiting germination of conidia of *Botrytis allii* at dilutions of 1 in 32. Two strains of *E. nigrum* received from Baarn (their no. 724 and '*E. neglectum*') were only slightly less active.

All strains grew very prolifically in stirred culture, the cultures being highly pigmented (orange-brown) and porridge-like in consistency. They were only filterable with difficulty and filtrates commonly set to a gel on standing. Production of antifungal material was also greater under these conditions; *E. purpurascens* (ACC. 1336), when grown on medium T with 10 % glucose, produced filtrates inhibiting *Botrytis allii* at 1 in 512.

Two strains were then selected for further study: *E. purpurascens* CMI. 68797 (ACC. 1336 in our culture collection) and one of the strains of *E. andropogonis* received from Baarn (ACC. 1334 in our collection). Because of the difficulty experienced in filtering stirred cultures, surface cultures were used in most work on the isolation of the active material, and in further studies on medium composition it was found that, for ACC. 1336, medium T with 5 % glucose ('Dextrosol', Corn Products Ltd.) was most productive, and, for ACC. 1334, Czapek-Dox or Raulin-Thom with 5 % glucose ('Dextrolact') was best.

Isolation of active material

The active principle was extracted from the surface culture fluids of both *E. purpurascens* (ACC. 1336) and *E. andropogonis* (ACC. 1334). After removal of the mycelium, the culture filtrate was adjusted to pH 2·0 and extracted with charcoal (15 g./l.). The charcoal was removed by centrifuging, air dried at room temperature and extracted with acetone. In a typical batch from ACC. 1336 on medium T, culture filtrate (42 l.) was extracted with charcoal (630 g.). Elution with acetone (500 ml.) gave at first an inactive dark solid (26·1 g.). Further elution with acetone (2 l.) removed the active compound, which separated as a yellow crystalline solid (33·2 g.). A further amount of less pure active compound (11·3 g.) was obtained by concentration of the eluates.

The active compound crystallized from aqueous dioxan in yellow needles, m.p. 228–229° (dec.). (Found: C, 55·1; H, 4·2; C-Me, 5·8 %. Calc. for $C_9H_8O_5$, C, 55·1; H, 4·1; C-Me, 7·1 %.) The literature m.p. for flavipin is 233–234° (dec.) (Raistrick & Rudman, 1956).

The molecular weight determination (crystals from acetone) showed space group Fdd2 and unit cell dimensions of $a = 10{\cdot}37$, $b = 10{\cdot}56$; $c = 30{\cdot}76$ Å. The crystals had d 1·56 g./cc., giving cell weight 3184·7 and molecular weight 199·0 ± 2 % (16 molecules per unit cell). (Calc. for $C_9H_8O_5$, M, 196·2.)

The penta-acetate hydrate and trimethoxyphthalide were both prepared as described (Raistrick & Rudman, 1956) and found to be identical with authentic specimens by mixed m.p. and comparison of the infrared spectra.

Biological activity of flavipin

At pH 3·5, flavipin almost completely prevented germination of *B. allii* conidia at 12·5 μg./ml.; this is in agreement with the observations of

Raistrick & Rudman (1956). Almost all other fungi tested were less sensitive to flavipin. At pH 3·5, aqueous solutions retained their antifungal activity for 22 days; at pH 4·5 or above, antifungal activity was rapidly lost. This instability may account for its negligible antibacterial activity *in vitro* in media at pH 7·0. Flavipin reduces the extension of pea stem sections at 100 μg./ml. but not at 10 μg./ml. It reduces cress root growth severely at 100 μg./ml. but scarcely at all at 10 μg./ml. It has no effect on the growth of pea seedlings when sprayed on, at 100 μg./ml. Thus it is actively antifungal but only slightly phytotoxic.

Discussion

Many species of *Epicoccum* have been described, but recent opinion favours the view that most of them really fall into the single species, *E. nigrum* Link (Ellis, 1956; Schol-Schwartz, 1959). Thus of our cultures, those received as *E. purpurascens* and *E. nigrum* all represent this one species. Recently, Schol-Schwarz (1959) has transferred the species previously known as *Cerebella andropogonis* Ces. to *Epicoccum*, as *E. andropogonis* (Ces.) Schol-Schwarz. It is interesting to note that we have found that this species, like *E. nigrum*, produces flavipin, confirming their close relationship.

In surface culture one of our organisms (ACC. 1336) produced three times the best yield of flavipin obtained by Raistrick & Rudman (1956) from *Aspergillus* spp.

We are indebted to Prof. J. H. Birkinshaw and Dr C. E. Stickings, London School of Hygiene and Tropical Medicine, for making available authentic specimens of flavipin as prepared by Prof. H. Raistrick, F.R.S., and Dr P. Rudman; to Dr P. G. Owston for the X-ray data; and to several colleagues for technical assistance.

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GROWTH OF ISOLATES OF *PHYTOPHTHORA FRAGARIAE* HICKMAN IN THE PRESENCE OF VARIOUS POLYPHENOLS

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(With 1 Text-figure)

Addition of certain polyphenols to a liquid or solid basal medium induced wide differences in the growth rate of seven isolates of *Phytophthora fragariae* Hickman, representing three pathogenic races. The presence of gallic, protocatechuic and chlorogenic acids, D-catechin and DL-tyrosine at a concentration of 0·001 M generally increased growth, whereas growth was restricted or inhibited by the same concentration of cinnamic acid, pyrocatechol and *p*-cresol. There was no similarity in the pattern of response to a given set of polyphenols either between isolates of the same pathogenic race, or between isolates from different pathogenic races; all isolates behaved differently, irrespective of source and pathogenicity. There were wide discrepancies between the patterns determined from measurements of radial growth on solid media and of dry weights of mycelia from liquid media.

Only very low levels of extracellular polyphenol oxidase activity could be detected in cell-free culture filtrates.

The isolation of a melanic pigment from the roots of strawberry plants affected by red-core root rot (Jarvis, 1956) indicated that profound changes in polyphenol metabolism occurred in the successfully parasitized host plant; the pigment was absent from unsuccessfully parasitized, i.e. resistant, roots and the possible role of certain polyphenols as factors in disease-resistance mechanisms was examined. In this paper is treated the behaviour in vitro of various isolates in the presence of certain polyphenols, some of which had previously been demonstrated in strawberry roots (Jarvis, 1956).

Differences in the utilization of polyphenols have often been demonstrated in fungi, particularly in wood-rotting species. Davidson, Campbell & Blaisdell (1938) used the Bavendamm (1928) reaction (growth in the presence of gallic and tannic acids) to divide 210 species of wood-decaying fungi into ten growth and colour reaction groups. Higuchi & Kitamara (1953) and Higuchi (1954) showed that while gallic and tannic acids were oxidized by both laccase and tyrosinase, α-naphthol was oxidized only by laccase and tyrosine and *o*-cresol only by tyrosinase. The Bavendamm reaction was thus divided into the laccase and tyrosinase reactions and the test substrates used in the differentiation of wood-rotting fungi were α-naphthol and tyrosine or *p*-cresol. Levisohn (1959) differentiated between strains of the root-infecting fungus, *Boletus scaber*, by, inter alia, a gum guaiac test and a tannic acid Bavendamm test for polyphenol oxidase activity.

Polyphenols have been shown frequently to be associated with disease resistance mechanisms, either directly or indirectly, and the literature has been recently reviewed by Allen (1959).

The amino acid nutrition of *Phytophthora fragariae* has been compared with that of other species of *Phytophthora* by Fleetwood-Walker (1955), while Davies (1959) found that three physiologic races of *P. fragariae* were remarkably similar in their utilization of amino acids. Both authors showed that calcium in a concentration of the order of 40 p.p.m. was an essential nutrient.

MATERIALS AND METHODS

Isolates

Seven isolates of *P. fragariae* from various strawberry varieties were used in this work and are listed in Table 1. They are characterized by their pathogenicity to certain indicator strawberry varieties and represent three pathogenic races.

Table 1. *Source and pathogenicity of isolates of* Phytophthora fragariae

(data from Montgomerie, 1960.)

Isolate no.	Source	Reaction on indicator variety			Pathogenic race no.
		Aberdeen	Auchincruive no. 11	Auchincruive Climax	
81	Huxley	−	−	−	1
78	Royal Sovereign	−	−	−	
72	Auchincruive no. 5	−	−	−	
71	Auchincruive no. 2	+	+	−	2
91	Auchincruive no. 1	+	+	−	
74	Auchincruive Climax	++	++	+++	3
93	Auchincruive Climax	++	++	+++	

−, No infection; +, slight infection; ++, moderate infection; +++, heavy infection.

Media

The isolates were maintained on oatmeal agar (Hickman, 1940), and except where otherwise stated, the basal medium used in nutrition experiments comprised sucrose 10 g./l., Oxoid Lab-Lemco 1 g./l., Oxoid bacteriological yeast extract 2 g./l., and Oxoid mycological peptone 5 g./l. Sucrose was chosen as the main carbohydrate source because McKeen (1956) had shown that a substance inhibiting growth of *P. fragariae* was formed when reducing sugars were autoclaved with glycine, a constituent of the peptone. Calcium in a sufficient concentration for growth is contained in the mycological peptone (Pierce, 1959).

Polyphenols were incorporated at the rate of 0·001M and, after adjusting to pH 6·5, the media were autoclaved at 15 lb. for 10 min., the liquid media in 12 oz. flat medicine bottles in aliquots of 30 ml.

Inoculum

Preliminary work established that size of the inoculum, i.e. the inoculum potential as defined by Garrett (1956), was very important in determining the growth rate of *P. fragariae* on agar media.

Table 2 gives the results of one experiment in which inocula of standard thickness, but of different diameter, were cut with cork-borers from the edge of 7-day colonies on basal medium agar and placed in the centre of basal medium agar plates. Radial growth was determined from measurement of six colony diameters.

Table 2. *Effect of inoculum diameter on growth of three isolates of* Phytophthora fragariae *on basal medium agar*

(Mean diameter of colony (mm.) less that of inoculum disk)

	72, race 1 Inoculum diameter (mm.)			78, race 1 Inoculum diameter (mm.)			74, race 3 Inoculum diameter (mm.)		
	3·5	7	10	3·5	7	10	3·5	7	10
After 3 days	0	0	0	0	0	0	0	0	2·0
5 days	0	2·6	3·3	0·7	2·5	5·7	5·5	7·2	10·0
7 days	0	6·0	14·0	2·3	10·0	14·5	12·7	16·3	18·0
10 days	0	19·5	26·2	13·0	23·6	28·6	26·2	29·2	29·2

Except where otherwise stated, disks 7 mm. in diameter and 2 mm. thick, taken from the edge of 7-day-old colonies on the basal medium agar, were used as standard inocula for both liquid media in medicine bottles and solid media.

Incubation and determination of growth

Medicine bottles containing inoculated media were incubated flat at 20° for 21 days, and the mycelium was then washed and dried to constant weight at 50° on tared filter papers. Cultures on agar media were also incubated at 20° and colony diameters were determined in two predetermined directions at right angles, after 10 days except where stated otherwise. The figures given in Tables 3–5 represent means of measurements on six colonies for each treatment.

EXPERIMENTAL RESULTS

Growth on non-polyphenol media

The results of experiments on the growth of *P. fragariae*, which had been carried out independently of those of Davies (1959), substantiate his findings that there were appreciable differences in growth rate between various isolates. Table 3 gives the results of an experiment in which three isolates of *P. fragariae*, representing two pathogenic races, were grown on media described by Law (1955) for work on the tyrosinases and laccases of wood-rotting fungi. The media were made up as follows:

I. 1 % Difco malt extract, 10 g./l.; agar, 15 g./l.

II. Glucose, 30 g./l.; potassium dihydrogen phosphate, 1 g./l.; marmite, 2 g./l.; agar, 15 g./l.

III. Glucose, 30 g./l.; aneurin 5 mg./l.; ammonium sulphate, 1 g./l.; potassium dihydrogen phosphate, 1 g./l.; copper sulphate, 0·1 mg./l.; agar, 15 g./l.

Table 3. *Growth of three isolates of* Phytophthora fragariae on *three non-polyphenol media*

(Mean diameter of colony (mm.) less that of inoculum disk (12 mm.).)

		72, race 1	78, race 1	74, race 3
Medium I	After 7 days	16·3	11·0	14·0
	10 days	22·5	18·3	15·5
	13 days	39·5	31·0	40·3
	17 days	46·5	54·6	47·0
Medium II	After 7 days	22·3	21·5	12·0
	10 days	29·0	24·0	15·0
	13 days	38·6	32·5	26·0
	17 days	39·5	42·0	37·3
Medium III	After 7 days	3·0	0·6	2·3
	10 days	3·5	0·6	2·5
	13 days	3·6	0·6	3·6
	17 days	5·0	0·6	4·3

The appearance of colonies of the three isolates was identical for any particular medium, but there were appreciable differences in growth rate. This is also apparent from the measurements of growth on basal medium agar recorded in Table 2.

Growth on polyphenol media

The method of Higuchi & Kitamara (1953) was used to ascertain the nature of the fungal polyphenol oxidase; DL-tyrosine and α-naphthol were incorporated into basal medium agar at a concentration of 0·001 M and diameters of colonies were determined 14 days after inoculation. The results are given in Table 4.

Table 4. *Growth of three isolates of* Phytophthora fragariae *in the presence of* DL-*tyrosine and α-naphthol*

(Mean diameter of colony (mm.) less that of inoculum disk.)

	With DL-tyrosine	With α-naphthol
72, race 1	46·3	0
78, race 1	68·7	0
74, race 3	69·7	0

None of the three isolates used in this experiment was able to utilize α-naphthol, and it can be assumed that there was no laccase activity. Isolate 72 showed a substantially lower growth rate in the presence of DL-tyrosine than the other two isolates, including one from the same pathogenic race.

The following polyphenols were then incorporated into the basal medium to give a concentration of 0·001 M: gallic acid, tannic acid (B.D.H.), protocatechuic acid, pyrocatechol, D-catechin, DL-tyrosine, caffeic acid, cinnamic acid, chlorogenic acid and *p*-cresol. Quantitative determinations of growth after 21 days in liquid and on solid media were made by the methods described above.

The growth of the various isolates in the liquid and solid basal media is given in Table 5. Mycelial dry weights are means of three replicates, and colony diameters are means of six replicates. In Fig. 1, growth of the isolates in the presence of the various polyphenols in both types of media is compared with growth in the basal medium. For this the figures have been taken from Table 5 and reduced to unity in each case.

Table 5. *Growth of seven isolates of* Phytophthora fragariae *in liquid and on solid basal media*

	Mean dry weight of mycelium in liquid medium	Mean diameter of colony (mm.) less that of inoculum disk on solid medium
72, race 1	169	41·6
78, race 1	141	23·7
81, race 1	150	31·0
71, race 2	126	13·0
91, race 2	168	49·0
74, race 3	200	31·0
93, race 3	70	56·7

It is clear from Table 5 that there were considerable differences in growth between the various isolates as determined in any one particular basal medium, and that, comparing growth on the two media (i.e. liquid and solid), there were large discrepancies between the two methods of determination. Fig. 1 also shows this type of discrepancy in determinations of growth in the presence of polyphenols.

It is also evident that each of the isolates is unique in its responses to the given set of polyphenols in either type of medium. There appears to be no resemblance in this respect between isolates from the same pathogenic race or from the same host variety, nor between isolates from different races and sources. In general the polyphenols depressed growth on solid media, except in the case of isolate 71 in the presence of chlorogenic acid, where growth was almost doubled. In many cases there was restriction or inhibition in both types of medium, particularly by pyrocatechol, cinnamic acid and *p*-cresol, although two or three, but not the same, isolates in each case were able to grow to some extent. Growth rates in liquid media were enhanced in most cases by the addition of gallic acid, protocatechuic acid, D-catechin, DL-tyrosine and chlorogenic acid.

Production of extracellular polyphenol oxidases

Three isolates of *P. fragariae* were grown in liquid polyphenol media and the cell-free culture filtrates were assayed for extracellular polyphenol oxidase activity by the method of Ponting & Joslyn (1948). Reaction mixtures comprised 3 ml. culture filtrate and 4 ml. 1 % pyrocatechol or DL-tyrosine in McIlvaine's buffer (McIlvaine, 1921) at two pH values, 6·0 and 7·4; as the substrate became oxidized, changes in colour were

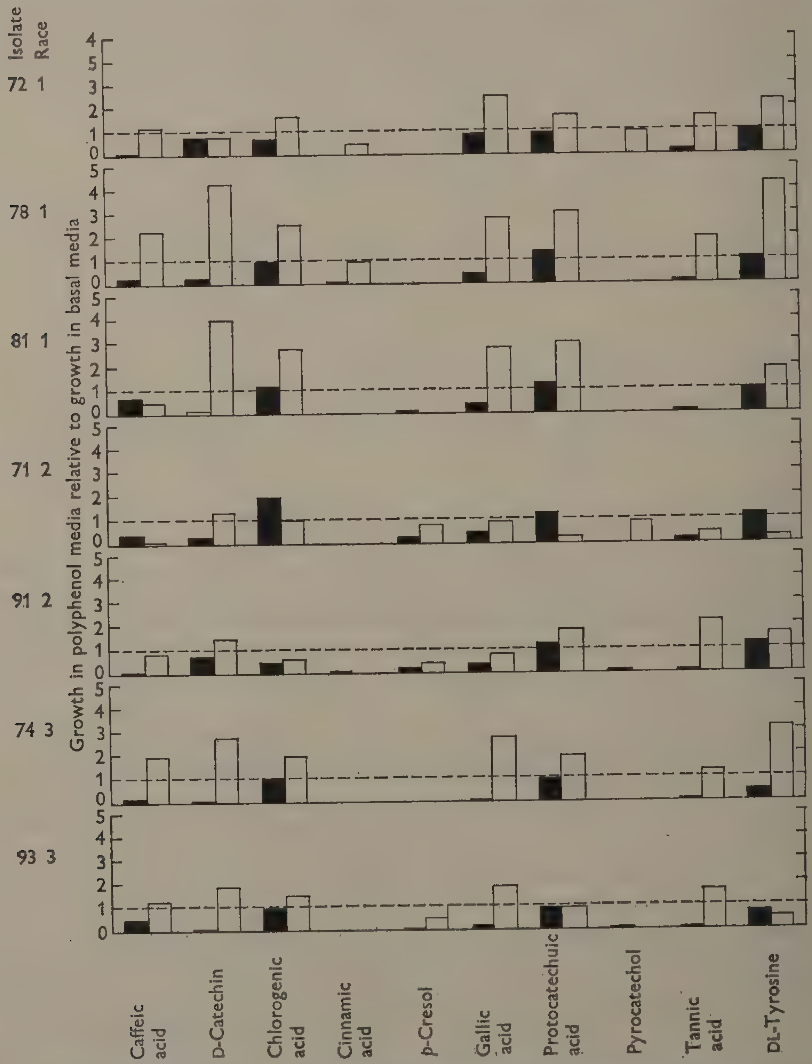

Fig. 1. Comparative growth of seven isolates of *P. fragariae* in the presence of various polyphenols in liquid (open histograms) and solid media (shaded histograms). The reference level of growth in liquid and solid basal media is given by the broken lines at unity.

determined at 470 mμ by Spekker absorptiometer after 1 hr. Activities in the culture filtrates were compared with that of a crude potato polyphenol oxidase preparation which was given the arbitrary value of 1000. Filtrates were examined from cultures of three ages, 17, 28 and 43 days. The enzyme activity found by this method was either extremely low or could not be detected, and the values recorded were nearly all below 5 % of that of the potato enzyme preparation. In many cases, mycelial dry weight fell at the end of the experiment, indicating that autolysis had occurred, but comparison of the ratios of enzyme activity units/mg. dry wt. showed no evidence that this ratio was constant with time for any particular isolate-polyphenol combination, or that more or less enzyme appeared in the medium with autolysis.

DISCUSSION

The fact that isolates of *P. fragariae* have widely differing tolerances to a given set of polyphenols appears to be of little phytopathological significance. There is no obvious resemblance in this respect between isolates originating from the same host variety or belonging to the same pathogenic race, and differences in pathogenicity cannot be explained in terms of tolerance in vitro to any of the polyphenols at the concentration used in these experiments. Strawberry roots have been shown to contain a number of polyphenols, including chlorogenic and caffeic acids, and at least one unidentified polyphenol which shows a seasonal variation (Jarvis, 1956), but it has not yet been possible to correlate the presence of these with resistance to pathogenic races of *P. fragariae*.

The discrepancies between the two methods of growth determination, i.e. of growth in liquid and solid media, are noteworthy, and recall similar discrepancies obtained with other fungi, for example, *Thielaviopsis basicola*, *Rhizoctonia solani* and, in particular, *Pythium ultimum* by Bateman & Dimock (1959). In all liquid cultures of *P. fragariae* the mycelium was largely submerged, and on agar media almost completely superficial, so that the growth differences observed may have resulted from differences in oxygen tension or in the rate of diffusion of metabolites in the two types of medium.

On the basis of behaviour in the presence of individual polyphenols it is possible to define new physiologic races, e.g. isolates 71, 91 and 93 may be said to constitute one race because of their relatively high tolerance of *p*-cresol, but when behaviour to the set of ten polyphenols used in these experiments is considered, it is evident that each isolate constitutes a single physiologic race. The position is complicated by the wide discrepancies in results obtained from solid and liquid media, and it is evident that the behaviour of *P. fragariae* in vitro in the presence of these ten polyphenols, at the given arbitrary concentration, is of no value as a criterion in defining pathogenic races.

I have pleasure in acknowledging the technical assistance of Miss K. S. Forbes, and the helpful discussions with Dr A. R. Wilson. Dr I. G. Montgomerie kindly supplied cultures of *P. fragariae*.

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A STUDY OF TOMATO ROOT SURFACE ORGANISMS ANTAGONISTIC TO *VERTICILLIUM ALBO-ATRUM*

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Fifteen organisms antagonistic to *Verticillium albo-atrum* were isolated from the surface of tomato roots, a green fluorescent species of *Pseudomonas* being the most frequent. The degree of antagonism of the isolates was determined and one isolate of *Pseudomonas* chosen for inoculation and population studies on roots in unsterilized, unamended soil. Inoculating the antagonist on to seed or roots of young tomato seedlings had only a temporary effect on the root surface microflora. Seedling inoculation with five of the antagonists had no effect on infection of tomatoes by *V. albo-atrum.*

In 1879, De Bary described some antagonistic interactions between micro-organisms (Waksman, 1945), but the possible importance of this phenomenon in disease control was not investigated. Hartley (1921) was one of the first to investigate the use of antagonistic organisms in the control of soil-borne diseases of plants. He inoculated sterilized soil with the pathogen *Pythium debaryanum* and then with cultures of several fungi and a species of bacterium, and reported that 'the parasitic activity of *P. debaryanum* decreased following the inoculation of the soil with various saprophytes'. Numerous papers since have reported the control of soil pathogens by antagonistic organisms when tested in sterilized soil, but in unsterilized soil little or no control has been obtained unless the soil has been amended to favour the growth of the antagonists (Wood & Tveit, 1955). Garrett (1955) has stressed this failure to obtain biological control of soil-borne diseases and considers that it might have been predicted from ecological considerations. In moist, unsterilized, unamended soil, all nutrients available are utilized by the micro-organisms already present, and the population remains approximately constant. All relevant evidence indicates that this biological equilibrium is not easily upset. Even if an introduced organism did become established in a soil at a level of population higher than in the uninoculated soil, there is still no guarantee that it would be antagonistic to a pathogen on or near the root surface, because this might not be a suitable substrate for the introduced organism.

The above criticisms may not apply if antagonists were isolated from the root surface. It is known that roots exude nutrients such as glucose, fructose and amino acids (Rovira, 1956), which could be utilized by an organism introduced on to the root surface, either by inoculating the root surface or by inoculating the seed. There is some evidence to support this view since Morrow, Roberts, Adams, Jordan & Guest (1938) reported that certain fungi antagonistic to *Phymatotrichum omnivorum*, when inoculated on to seed or added to exposed roots of cotton, 'became established in the rhizosphere. The introduced organisms [were recovered] in appreciably greater numbers from inoculated plants than from those not receiving inoculum.' They presented no evidence that the incidence of disease was affected by such treatment.

Seed inoculation of non-legumes with various species of bacteria is practised widely in the U.S.S.R. (Cooper, 1959) and some workers have claimed that such treatment can reduce the incidence of disease (Bel'tyukova, 1955; Khariton & Geller, 1958), but they give little information on the fate of bacteria after seed is sown.

In the present work, an organism antagonistic to *Verticillium albo-atrum* and isolated from the surface of tomato roots was inoculated on to seeds and roots of young tomato seedlings and its population measured at weekly intervals. The effect of inoculating plants with various antagonists on infection of tomatoes by *V. albo-atrum* was also studied.

MATERIALS AND METHODS

Soils. During the investigation three soils were used. Their origin was as follows:—

(1) Cambridge Botanic Garden soil—a light loam, pH *c.* 7·2; moisture holding capacity (M.H.C.) 50 ml./100 g. air-dry soil.

(2) Kettering loam—a medium loam with a fairly high organic and total nitrogen content; pH 5·0–6·0; M.H.C. 65–70 ml./100 g. air-dry soil.

(3) John Innes compost—prepared from Kettering loam (7 vol.), which was steam sterilized before being mixed with peat (3 vol.) and sand (2 vol.); pH 6·3; M.H.C. 70 ml./100 g. air-dry compost.

Culture medium. A medium containing 0·1 % fructose, 0·1 % glucose, 0·1 % casamino acids and 2 % agar in soil extract (prepared by autoclaving 1 kg. soil with 1 l. water followed by filtration) was used throughout. The reaction of the medium was adjusted to pH 7·0.

Isolation of root surface organisms. To isolate organisms from the root surface of tomato plants growing in unsterilized soils, a technique was required which would separate root surface organisms from those in the surrounding soil. Harley & Waid (1955) isolated root surface fungi by a serial washing technique and in the present work the possibility of isolating tomato root surface organisms by this method was investigated.

Tomato plants, 3 weeks old, were removed from Botanic Garden soil and the roots were dipped in sterile water to remove adhering soil particles and then cut into 1 cm. lengths; two lots of ten pieces were selected and subjected separately to twenty serial washings with 10 ml. aliquots of sterile distilled water in McCartney bottles. For each washing the roots were shaken for 2 min., using a 'Microid' wrist-action shaker. Dilution series of the water used for the second, fifth, tenth, fifteenth and twentieth washings were made and 0·2 ml. aliquots placed in sterile Petri dishes. Each plate was poured with 20 ml. of cool molten agar and incubated for 2 days. The washed roots were homogenized in a glass homogenizer with 10 ml. water, and after suitable dilution 0·2 ml. aliquots of the homogenate were also plated out and incubated. The results are given in Table 1.

Since the organisms remaining on the roots after twenty serial washings must have been in close association with the roots and unlikely to be contaminated with soil-inhabiting organisms, washing ten 1 cm. lengths of root in twenty changes of sterile water was adopted as standard practice for studying populations of root surface organisms. Occasional bacterial colonies spread over the surface of the agar, and to prevent this the agar was added to the homogenate in two lots of 10 ml., the second lot being added after the first had solidified.

Table 1. *Calculated numbers of organisms washed from, and remaining on, 1 cm. length of tomato root during serial washings*

Washing water					Washed root surface
2nd wash	5th wash	10th wash	15th wash	20th wash	
> 10,000	1250	720	275	152	3600

Table 2. *Isolation of organisms antagonistic to* Verticillium albo-atrum *from tomato root surface*

Time of sampling after sowing (weeks)	Total numbers of organisms and antagonists on six plates					
	Botanic Garden soil		John Innes compost		Kettering loam	
	Organisms	Antagonists	Organisms	Antagonists	Organisms	Antagonists
3	793	4	20	0	203	1
4	1822	1	205	0	123	0
5	—	—	517	0	1380	2
6	638*	0	1021	5	1594	3
Total	3253	5	1763	5	3300	6

* 1/40 dilution; all other samples were at 1/10 dilution.

DETECTION AND ISOLATION OF ANTAGONISTS

Tomato plants were grown from seed in the three soils in pots, and the presence of antagonists on the root surface was determined after 3, 4, 5 and 6 weeks. One root sample containing roots from two plants was used for each determination; two dilution series were made and three plates were prepared from each dilution. Plates were incubated for 2 days and then sprayed with a spore suspension of *Verticillium albo-atrum*,* using the type of atomizer described by Wilska (1947). Plates were incubated for another 3 days and then examined for zones of inhibition in the otherwise uniform growth of *V. albo-atrum*. The number of inhibition zones and the total number of organisms on the plates were counted (Table 2). The organism associated with each zone was isolated, unless two or more antagonists on a plate appeared identical. The antagonists were purified and kept for further study.

The organisms on the plates were predominantly bacteria, with a few actinomycetes and practically no fungi. This might be explained by the short incubation period. There was a considerable difference in the number of organisms obtained from the three different soils, particularly in the samples taken after 3 and 4 weeks, and the ratio of total number of organisms to antagonists varied markedly between samples. For instance, in the 3-week sample from Botanic Garden soil there were 739 organisms and 4 antagonists giving a ratio of 185:1, whereas the sample taken from the same soil after 4 weeks gave 1822 organisms and only 1 antagonist.

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* The presence of organisms antagonistic to *Fusarium oxysporum* f. *lycopersici* was also determined in a duplicate series of plates, but further tests with this pathogen were discontinued.

From all samples, fifteen organisms antagonistic to *V. albo-atrum* were isolated and purified and their degree of antagonism measured. Each antagonist was streaked across three plates, near the periphery, incubated for 2 days and then a spore suspension of *V. albo-atrum* was streaked at right-angles to it (Patrick, 1954). Table 3 records the distance between the pairs of organisms after 2 days.

Table 3. *Degree of antagonism of isolates to* Verticillium albo-atrum

Isolation no.	Soil from which isolated	Identity	Distance between antagonists and test organism (mm.)
T1/1	Botanic Garden	*Pseudomonas* sp.	28
T1/2	Botanic Garden	*Pseudomonas* sp.	5
T1/3	Botanic Garden	*Pseudomonas* sp.	30
T1/4	Botanic Garden	*Pseudomonas* sp.	4
T1/5	Kettering loam	*Pseudomonas* sp.	29
T1/6*	Botanic Garden	*Pseudomonas* sp.	30
T1/7*	Botanic Garden	*Pseudomonas* sp.	28
T1/8*	Botanic Garden	*Pseudomonas* sp.	28
T2/1	Botanic Garden	*Pseudomonas* sp.	32
T3/1	Kettering loam	Unidentified bacterium	6
T4/2	John Innes compost	*Pseudomonas* sp.	28
T4/3*	Botanic Garden	*Streptomyces* sp.	5
T4/4	John Innes compost	Unidentified bacterium	2
T4/5	John Innes compost	Unidentified bacterium	1
T4/6	Kettering loam	*Streptomyces* sp.	7

* Originally isolated as an antagonist of *Fusarium oxysporum* f. *lycopersici*.

Of the fifteen antagonists isolated, ten were identified as *Pseudomonas* sp., two as *Streptomyces* spp. and three were unidentified bacteria. The *Pseudomonas* isolates produced a green, diffusible fluorescent pigment in the medium described by King, Ward & Raney (1954) for the production of fluorescin. From cultural examination they appeared to be a homogeneous group of organisms but as antagonists they fell into two distinct groups; those in one group, consisting of T1/1, T1/3, T1/5, T1/6, T1/7, T1/8, T2/1 and T4/2, were strongly antagonistic, whereas those in the other group (T1/2 and T1/4) were weak antagonists. Isolate T2/1 showed the highest degree of antagonism and was selected for further study. It had the following biochemical properties: no acid in purple lactose agar; acid only in lactose, sucrose and maltose (in peptone-free medium); NO_2 not formed from NO_3; gelatine liquefied; green fluorescence in meat-infusion agar; no pyocyanin in glycerol-peptone agar.

INOCULATION OF TOMATOES WITH *PSEUDOMONAS* SP.

A bacterial suspension of isolate T2/1 was prepared by adding ten 48 hr. cultures on agar slopes to 250 ml. sterile water to give 7×10^8 cells/ml. Tomato seed and the roots of 25-day seedlings were inoculated by immersing for 5 min. in the bacterial suspension, and seed and seedlings were then planted in Botanic Garden soil. Uninoculated seeds and seedlings were used as controls. The population of organisms and antagonists on the roots was determined at weekly intervals, commencing 1 week after inoculation. Two plant and two seed replicates, two separate dilution series and two plates for each dilution were used for every determination. The calculated number of organisms on 1 cm. lengths of root and the ratio of organisms to antagonists (O/A) are given in Table 4.

Table 4. *Calculated numbers of organisms on surface of 1·0 cm. lengths of tomato root, and ratio of organisms to antagonists (O/A)*

	After									
	1 week		2 weeks		3 weeks		4 weeks		5 weeks	
	No. organisms	O/A	No. organisms	O/A	No. organisms	O/A	No. organisms	O/A	No. organisms	O/A
Seeds										
Control	2,850	134	1,550	∞	1,188	∞	—	—	—	—
Inoc.	813	15	875	∞	1,175	∞	—	—	—	—
Plants										
Control	5,944	1,156	8,925	211	7,500	∞	10,625	∞	14,563	∞
Inoc.	25,925	11	39,188	14	44,813	15	22,250	230	15,438	∞

With seed inoculation, there was a much lower O/A ratio after 1 week on roots from inoculated than from uninoculated seed, but after 2 and 3 weeks no antagonists were detected from either treatment. It would appear that inoculation of tomato seed with isolate T2/1 does not favour colonization of roots by this particular organism.

With root inoculation of young seedlings, there was a high population of antagonists on the roots after 1, 2 and 3 weeks, but this was followed by a sudden drop in number of antagonists, and after 5 weeks none was detected. There was a highly significant difference between the total number of organisms on the roots of control and inoculated plants. Inoculation of roots with isolate T2/1 at the level used caused a slight browning of the roots, indicating tissue damage either by the organism itself or by metabolic products from it, and it is thought that the damaged tissue supported the growth of a much higher population of organisms than did the uninoculated, undamaged roots. Again it would appear that inoculation of tomato seedlings had only a very temporary influence on the microbial flora of the root surface.

The increase in the number of root surface organisms with time of sampling in the control series is almost certainly a reflexion of the sampling technique, which involved unit lengths of root. The older the plant, the greater will be the surface area of these unit lengths of root and therefore the greater the number of root surface organisms. An increase in the number of senescent root hairs would also increase the microbial count with age.

Although the effect of inoculation was so transient, an experiment was designed to determine if inoculation of tomato seedlings with antagonists had any noticeable effect on infection by *V. albo-atrum*. The three soils were inoculated with a spore suspension of *V. albo-atrum* and 24 hr. later seedlings inoculated separately with antagonists T1/5, T1/8, T2/1, T4/2 and T4/6 were planted in the soils, the effect of each organism being tested in the soil from which it had been isolated. None of the antagonists had any influence on infection. After 5 weeks all plants growing in soils infested with *V. albo-atrum* were infected.

DISCUSSION

The antagonist of *V. albo-atrum* most frequently isolated from the surface of tomato roots was *Pseudomonas* sp. This is in contrast to studies of non-rhizosphere soils. Stessel, Leben & Keitt (1953), for example, found that 80 % of the antagonists isolated from forty soil samples were actinomycetes. Although well known as antagonists of other bacteria (Waksman, 1945), species of *Pseudomonas* have rarely been recorded as fungal antagonists. Wood (1951), however, listed *P. fluorescens* as showing striking antagonism to *Botrytis cinerea*, and recently Roth (1959) showed that three strains of *Pseudomonas* isolated from barley seed were antagonistic to several fungi.

The strain T2/1, selected for detailed study, was a fast growing organism and occurred naturally on the surface of tomato roots, two characters which should favour colonization of roots following seed or root inoculation. Nevertheless, inoculation of seed or roots of tomato with this organism had only a very temporary effect on the root surface population; either the introduced bacteria failed to grow or, if they grew, they failed to spread along newly formed roots. The ratio of inoculated roots to new roots would decrease with every sample and the chance of including a piece of inoculated root in the sample would be correspondingly less. Another possible interpretation is that the introduced organism lost its ability to antagonize *V. albo-atrum* and so its presence would not be revealed by the method employed. These results are similar to those obtained by Clark (1948) who followed the population of *Azotobacter* spp. on inoculated tomato roots and found a steady decrease following inoculation. Clark concluded, however, that *Azotobacter* spp. are not particularly adapted to a rhizosphere environment, whereas green fluorescent *Pseudomonas* spp. are (Clark, 1940). However the antagonistic strains probably form only a low percentage of the total population of *Pseudomonas* spp. on the root surface and may not be good colonizers.

The failure to obtain increased numbers of *Pseudomonas* sp. on the surface of tomato roots following inoculation does not necessarily refute Russian work on inoculation of non-legumes, but it indicates that further detailed quantitative bacteriological studies are desirable.

The present work was carried out at the Botany School, Cambridge, during a period of study leave from the University of Adelaide. I wish to thank Dr W. J. Dowson and Dr A. C. Hayward for biochemical tests with some of the bacterial antagonists.

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HUMICOLA STELLATUS SP.NOV., A THERMOPHILIC MOULD FROM HAY

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(With Plate 26 and 2 Text-figures)

Humicola stellatus sp.nov., a thermophilic mould found in mouldy hay from England and Wales, is described. *Monotospora lanuginosa* (Griff. & Maubl.) Mason is transferred to *Humicola*.

Humicola stellatus sp.nov. (Pl. , text-fig. 1)

Hyphae 1–2·8 μ diam., thermophilae, 40° C. lente crescentes. Conidiophora solitaria, lateralia, 3·0 μ (0·5–11 μ) longa, ab hyphis rectis aeriis oriunda. Conidia recentia—subglobosa et hyalina; matura-clara brunnea et saepe cadentia, levia, 4–6(–8) angustitrunculosis rotundatis protuberationibus (incluso hilo) stellata; minima diametros 5·3 μ (3·5–6·8 μ), maxima (trans protuberationes) 7·6 μ (4·5–10·5 μ).

Habitat faenum gramineum in Anglia et Cambria. Typus Herb. I.M.I. 77024 est.

The type specimen, isolated from hay from Rothamsted, Harpenden, Herts., is deposited in Herb. I.M.I. as no. 77024. Type material has also been deposited in Herb. Kew; Herb. DAOM, Ottawa. Cultures have been placed in the C.M.I. culture collection and at C.B.S., Baarn.

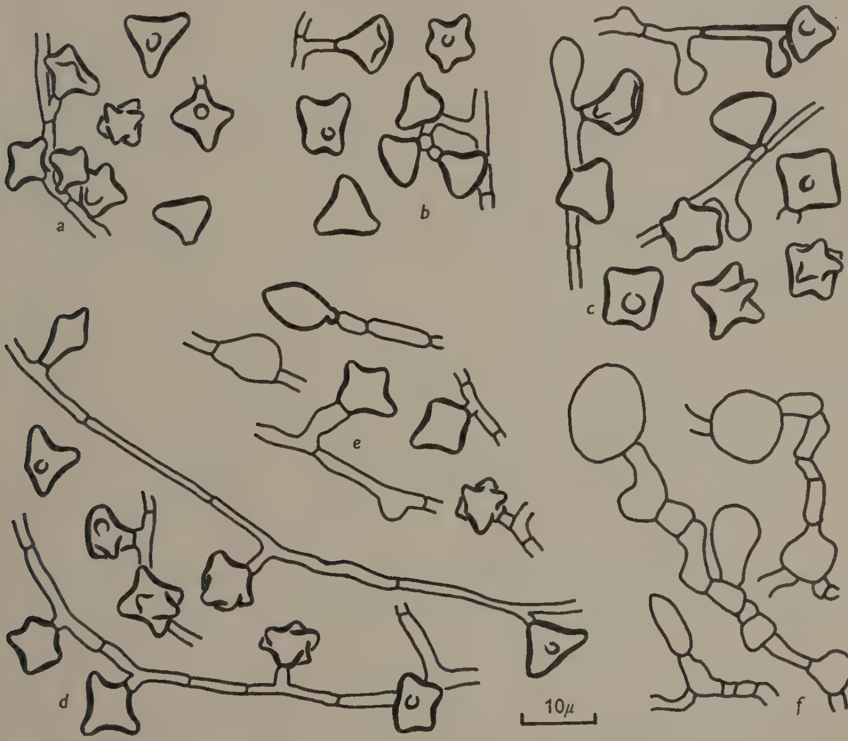
The specific epithet refers to the shape of the spores.

In 1959, during the early stages of a study of dust from mouldy hay, small irregularly shaped brown spores were seen in several samples. It was not until some 'hay' with a moisture content of 42 % was incubated in a sealed tin at 40° C. that the fungus was seen in quantity. From this incubated hay the fungus was isolated on hay infusion agar (20 g. hay soaked in 1 l. water overnight, strained, and 2 % agar added) at 40° as a dematiaceous mould apparently hitherto undescribed. It has since been found in numerous samples of mouldy hay that have obviously heated spontaneously during storage; the samples came from places as far apart as Cornwall, North Wales, Cumberland and Hertfordshire. After seeing a culture of this organism, Mr P. K. C. Austwick, Central Veterinary Laboratory, Weybridge, found sketches (drawn by him in July 1952) of some spores of an identical fungus from a sample of very mouldy hay sent to him from near Wolverhampton.

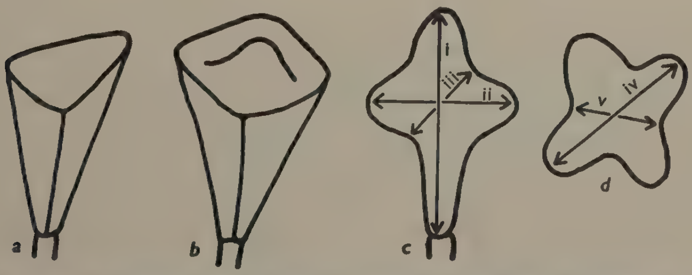
One spore occurred on a Hirst spore trap slide exposed on the afternoon of 25 July, 1958, at Imperial College Field Station, Silwood Park, Ascot, Berks.

Material was examined by Dr M. B. Ellis and Mr J. J. Elphick at the Commonwealth Mycological Institute, Kew, and by Dr S. J. Hughes, Plant Research Institute, Canada Department of Agriculture, Ottawa, all of whom agreed in referring it to the genus *Humicola* Traaen (1914).

On hay, its natural substrate, *H. stellatus* forms a superficial network of hyaline, septate hyphae, 1–2·8 μ diam. Aleuriophores arise on long aerial hyphae, are solitary, lateral, 1·2–2·8 μ diam. and 3 μ(0·5–11 μ) long, occasionally separated from the basal cell by a septum. Aleuriospores (Text-fig. 1) are borne singly and terminally on the aleuriophores; they



Text-fig. 1. *Humicola stellatus* sp.nov. Developing and mature spores grown at 40°. *a*, From hay, Rothamsted 1959; *b*, from hay, Rothamsted 1960; *c*, from 6-month-old culture on 'Oxoid' nutrient agar; *d*, from 5-day-old culture on 'Oxoid' nutrient agar; *e*, from Czapek+peptone agar; *f*, from Czapek+sucrose agar.



Text-fig. 2. *Humicola stellatus* sp.nov. Diagram showing basic shape of spores. *a*, With four projections; *b*, with six projections; *c*, side view of spore with six projections; *d*, top view of spore with six projections. (i–v, positions of measurements.)

are subglobose and hyaline when young, clear dark brown and stellate when mature (Text-fig. 2), and they often become detached. The spore wall is smooth and is thinner towards the tips of the 4–6(–8) projections (including the hilum). Table 1 shows the mean, standard deviation and range of sizes of spores from five different samples (each of twenty-five spores) measured in the five positions (i–v) of Text-fig. 2.

Table 1. *Measurements of spores of* Humicola stellatus

	View (Text-fig. 2*c*)		
Specimen	Length, μ (i)	Width, μ (ii)	Minimum, μ (iii)
Hay, Cornwall	7·2 ± 0·69 (5·5–8·5)	7·2 ± 0·92 (5·5–9·0)	5·4 ± 0·94 (4·0–6·5)
Hay, Rothamsted 1959	6·6 ± 0·90 (5·0–10·0)	7·2 ± 0·87 (6.0–10.25)	4·9 ± 0·46 (4·0–5·75)
C67 (*a*)	8·0 ± 0·22 (6·5–9·0)	8·2 ± 0·77 (7·0–9·5)	5·8 ± 0·44 (5·0–6·75)
C67 (*b*)	8·0 ± 0·99 (5·25–9·5)	8·0 ± 0·74 (6·5–10·0)	5·6 ± 0·71 (4·0–6·75)
Hay, Rothamsted 1960	6·4 ± 0·90 (5·0–9·0)	6·9 ± 0·80 (5·0–8·5)	4·8 ± 0·42 (4·0–5·5)
Mean	7·2	7·5	4·9

	View (Text-fig. 2*d*)	
Specimen	Maximum, μ (iv)	Minimum, μ (v)
Hay, Cornwall	8·2 ± 0·78 (7·0–10·0)	5·5 ± 0·55 (4·75–6·75)
Hay, Rothamsted 1959	7·7 ± 0·81 (6·5–9·0)	5·0 ± 0·10 (4·5–5·75)
C67 (*a*)	8·8 ± 0·65 (8·0–10·5)	5·8 ± 0·70 (4·5–7·5)
C67 (*b*)	9·0 ± 0·26 (7·5–10·5)	5·6 ± 0·41 (5·0–6·5)
Hay, Rothamsted 1960	7·4 ± 0·86 (5·5–9·0)	4·9 ± 0·55 (3·5–5·75)
Mean	8·2	5·3

C67—6-month-old culture grown on 'Oxoid' nutrient agar for 14 days at 40°: (*a*) mounted in glycerine jelly; (*b*) mounted in lactophenol.

Table 2. *Growth rates (increase in diameter per day in mm.) of colonies of* Humicola stellatus

	40°			24°		
pH ...	4·5	6·5	8·5	4·5	6·5	8·5
'Oxoid' nutrient agar (mm.)	3·5	3·3	2·5	0·6	0·5	0·4
Czapek + sucrose agar (mm.)	1·0	0·7	0·2	0·2	0·2	0·3
Czapek + peptone agar (no sugar) (mm.)	3·1	2·0	1·4	0·5	0·3	0·2

H. stellatus grows and sporulates well, forming a dark colony, on hay infusion agar and other media containing peptone or beef extract. On media containing only salts and sugars it grows more slowly, forms a pale colony, sporulates sparsely and produces hyaline chlamydospores in the hyphae. Its optimum temperature for growth is 40°; it grows very slowly at 24° and it survives without obviously growing at 50°. On 'Oxoid' nutrient agar at 40° the colony has a black reverse with a slightly paler edge; the surface is dark brown to black with a grey velvety sheen (Pl. 26, figs. 1–9). Growth is increased by lowering the pH (Table 2). Tests made by growing the fungus on strips of blotting paper in different liquid media showed it was not cellulolytic. The spores do not germinate readily;

those that have been seen germinating were immature and produced a germ tube from the scar of attachment to the aleuriophore.

As this fungus was found in hay associated with some diseases in animals, Mr P. K. C. Austwick kindly did pathogenicity tests. Three weeks after inoculating it intravenously into mice he could not recover it from any of the organs, but 3 weeks after inoculating intraperitoneally into guinea-pigs he found some of the original inoculum unchanged and viable in the peritoneal cavity.

Monotospora lanuginosa (Griff. & Maubl.) Mason is very similar to *H. stellatus*; it also occurs in hay, is thermophilic and non-cellulolytic, the aleuriospores are produced in the same manner and are fairly easily detached; no phialospores have been found. It differs in having globose spores with thick, rough walls, and in growing and sporulating well on the usual mycological media. The two genera, *Monotospora* and *Humicola*, have been confused for many years (Mason, 1933, 1941). *Monotospora* was erected by Corda (1837) for the single species, *M. toruloides*, but authentic material of the species in Herb. Corda shows no fungus which can definitely be related to Corda's description. Saccardo (1880) excluded *M. toruloides* from his concept of *Monotospora*, regarding *M. sphaerocephala* Berk. & Br. as the type species. Traaen (1914) established *Humicola* for *H. fuscoatra* and *H. grisea* isolated from Norwegian soil. These species produced aleuriospores in the same manner as *H. stellatus* and *M. lanuginosa*, but they occur in soil, are mesophilic, cellulolytic, and have phialospores; their aleuriospores do not readily become detached and are often described as chlamydospores. As *Monotospora* Corda is not definable, it seems that *Humicola* Traaen is the most suitable genus for *M. lanuginosa* and this species is therefore transferred as

Humicola lanuginosus (Griff. & Maubl.) comb.nov. (Basionym: *Sepedonium lanuginosum* Griffon & Maublanc 1911, in *Bull. Soc. mycol. Fr.* **27**, 70, figs. 1–3.)

I thank Mr D. H. Boalch for the Latin translation, Mr P. K. C. Austwick, Dr M. B. Ellis, Mr J. J. Elphick and Dr S. J. Hughes for helpful discussion, and Mr V. Stansfield and Mr F. D. Cowland for the photographs.

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EXPLANATION OF PLATE 26

Humicola stellatus sp.nov.

Figs. 1–6. 10-day-old cultures at pH 7·5 (× ½)

Fig. 1. On malt extract agar at 40°.
Fig. 2. On the same agar at 24°.
Fig. 3. On potato-dextrose agar at 40°.
Fig. 4. On the same agar at 24°.
Fig. 5. On 'Oxoid' nutrient agar at 40°.
Fig. 6. On the same agar at 24°.

Figs. 7–9. 11-day-old cultures at pH 6·5 and 40° (× ½)

Fig. 7. On 'Oxoid' nutrient + sucrose agar.
Fig. 8. On Czapek agar (without sugar).
Fig. 9. On Czapek + peptone agar (without sugar).
Fig. 10. Surface view of culture on nutrient agar (× 107).
Fig. 11. Mature spores (× 400).
Fig. 12. Spores in different stages of development (× 400).

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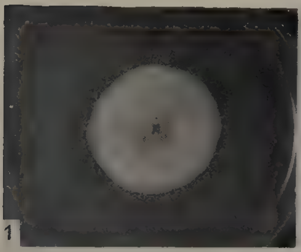
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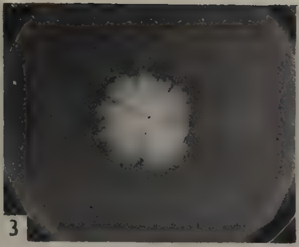
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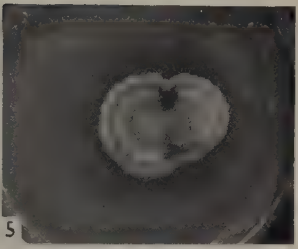
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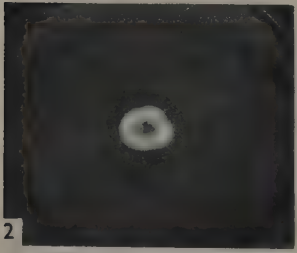
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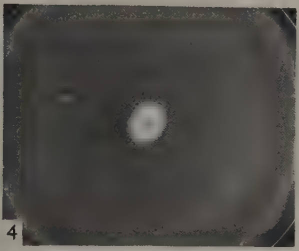
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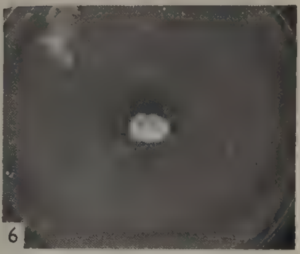
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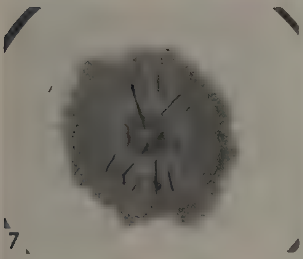
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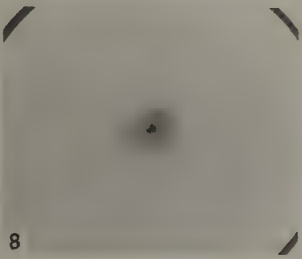
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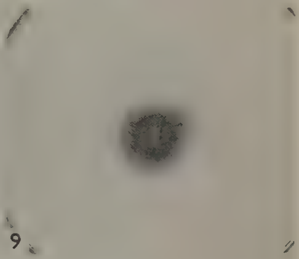
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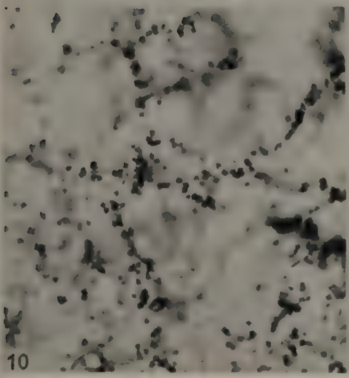
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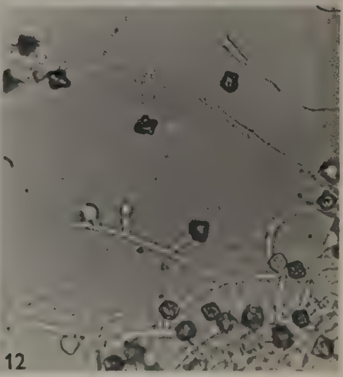
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MORPHOGENESIS, FUNGISTASIS AND CULTURAL STALING IN *FUSARIUM OXYSPORUM* SNYDER & HANSEN

By DAVID PARK

Botany Department, University of Manchester

(With Plate 27 and 5 Text-figures)

Morphogenesis in agar and liquid cultures can be explained by the accumulation in the medium of a single substance. In high concentrations the substance inhibits germination and growth, and causes autolysis. The substance is labile under normal laboratory conditions, its concentration in the culture being maintained by continued activity of the fungus even in apparently inhibited cultures. Cellophane film ameliorates its effect on the fungus. Other stylosporic fungi examined showed the phenomena found in *Fusarium oxysporum*, but fungi able to produce massive sporophores did not.

There has for some years been an interest in the toxicity of soil for fungi. The effect has been attributed by some workers to specific antibiotics; others have argued that the production of the effect is general to many of the organisms in soil. Park (1960) has compared the toxicity of soil with the condition of a staled fungal culture. Staling in a fungus has here been re-examined, better to understand the biology of fungi in soil.

MATERIALS AND METHODS

An isolate of *Fusarium oxysporum* Sny. & Hans., obtained from soil during routine isolations, and with no unusual cultural features was used.

The culture medium had the composition: glucose, 0·7 g.; $MgSO_4 . 7H_2O$, 0·5 g.; KH_2PO_4, 0·2 g.; NH_4NO_3, 0·1 g.; distilled water to 1 l. The pH of this medium was 4·4. When used as solid medium, 15 g. agar was added before sterilization. Petri dishes were poured with 15 ml. medium. Liquid medium was used in 20 ml. batches in 100 ml. wide-necked flasks. Cultures were incubated in conditions of alternating light and dark at 24–25° C.

STALING ON AGAR

Colonies after the first 2 days showed a regular pattern of differentiation in which seven zones were distinguished, measuring from the margin towards the centre.

(1) 0–200 μ. Broad (5–6 μ. diam.), straight marginal hyphae, orientated away from the inoculum. Little branching.

(2) 200–700 μ. Branching; branches less broad (4–5 μ diam.), turning in the direction of open areas (areas not isolated by hyphae from the marginal agar as seen from above), then showing straight growth.

(3) 700 μ–3 mm. Branching resulting in closed areas of agar as seen

from above. Laterals in these areas narrow (*c.* 3 μ diam.) and meandering. Hyphal loops and coils develop.

(4) 3–4 mm. Short laterals abstrict microconidia successively to form small groups of spores.

(5) 4–8 mm. Start of autolysis in the broad primary hyphae and in some of the straight laterals.

(6) 8–11 mm. Terminal and intercalary chlamydospores develop in narrow meandering hyphae.

(7) 11 mm.–centre. Much autolysis of all types of hyphae and a low density of staining hyphae. Numerous chlamydospores and groups of microconidia, not near any living hyphae.

The rate of growth of these cultures was 5 mm./day. Thus during the first 14 hr. from colonization of an area, there is a progressive maturation from broad, straight juvenile hyphae to narrow, meandering hyphae producing conidia on short laterals of limited growth. After 30 hr., autolysis of the colonizing hyphae starts, after 38 hr. chlamydospores begin to develop, and after 2 days there is a very low density of active fungus and the area can be considered staled.

The stages described above were observed in space, and the time intervals inferred. Observations at intervals of time were made from plates of agar seeded throughout with about 3000 microconidia per plate, and are summarized here.

18 hr. Germination normal. Germ-tubes 30–70 μ, some showing branching.

41 hr. Colonies 0·7–0·8 mm. diam., mostly below the agar surface and not contiguous. Marginal hyphae narrow and meandering, and laterals commonly recurving even in open areas of colonies. Microconidial production seen near centre of each colony. Near colony centre are branches with swollen tips. No autolysis.

55 hr. Colonies contiguous, but hyphae less dense between than within colonies. No further conidial production. Terminal chlamydospores common. Autolysis of hyphae, particularly near centre of colonies.

From this time on, up to the seventh day, there was little change, apart from an increase in the amount of autolysis. Very few microconidia had been produced, and they were all submerged, the colonies having originated from inocula below the agar surface. Staling is normally considered a static condition of over-maturity, yet on the seventh day microconida and hyphal loops and coils were seen on the surface where none had been recorded before. On the eighth and ninth days groups of conidia on the surface increased in frequency, and moreover pyriform chlamydospores appeared below the agar where only spherical ones had been found before. These observations suggest that the fungus is active in respect of hyphal growth and conidium and chlamydospore formation even in staled cultures, in which the overall hyphal density is low and autolysis predominates.

It will be noted that the timing of events differed in the two types of cultures, and also that in the seeded plates broad marginal hyphae showing directional orientation were not observed and that conidial production was greatly curtailed in the initial stages. The more uniform accumulation of staling substances here as opposed to the centrifugal accumulation in the cultures starting from a point inoculum are believed to contribute to these differences.

Staled fungal cultures have additional interest in that the spores produced in them remain ungerminated, although in *F. oxysporum* conidia and chlamydospores germinate readily when transferred from the staled culture to distilled water. That substances in the staled agar inhibit germination was demonstrated by transferring slabs of 14 day staled agar to blocks of similar surface area of distilled water agar to provide a diffusion sink. After 18 hr. on sinks 5–9 mm. deep, the staled agar contained much meandering non-autolysed mycelium producing micro- and macroconidia on side branches, and it was difficult to determine whether germination had occurred. After the same time on shallow sinks (1·5–3·5 mm.), conidium and chlamydospore germination were clearly seen, the germ-tubes being short and often producing microconidia from their apices.

The effect of dilution on fungal activity in a staled culture was more clearly demonstrated by cutting out, from near one side of a 9-day seeded plate, a trough 5 × 20 mm. and filling it with sterile distilled water. After 24 hr. the area on the margin of the trough was compared with that on the opposite side of the plate by counting frequency of stainable hyphae in 500 quadrats of area $6{\cdot}25\,\mu^2$, and by counting the number of conidia in twenty-five quadrats of area $0{\cdot}09$ mm.^{2} (Table 1). In the area subjected to dilution, hyphal frequency was almost 50% greater than in the untreated area, and conidial numbers showed a more than sevenfold increase.

Table 1. *Effect of dilution on hyphal frequency and conidial numbers in staled agar*

	Margin of trough	Untreated area
Hyphal frequency, $6{\cdot}25\,\mu^2$ quadrat	44	31
Mean no. conidia, $0{\cdot}09$ mm.^{2} quadrat	6·48	0·88

Since the staling effect is positive, due to the accumulation of substances in the medium, rather than negative, due to the removal of nutrients, attempts were made to induce staling phenomena at the juvenile margin of centrally inoculated growing cultures by adding blocks of staled agar and by filling Oxford cups with staled liquid medium. All the attempts were unsuccessful. These results are best interpreted in the light of experiments to be described below.

Staling in liquid culture

The cultures were inoculated with a suspension of spores and examined at intervals, by removing samples and mounting for microscopical observation.

3 days. Colonies *c.* 5 mm. diam. Hyphae of even width, evenly staining. Micro- and macroconidia being produced.

5 days. Colonies up to 14 mm. diam. Hyphae mostly granular and irregularly-staining. Some autolysis. Many apices sympodially branched. Ungerminated conidia common.

7–17 days. Increase in the amount of hyphal autolysis; decrease in the number of robust apices, most apices becoming autolysed or very attenuate. Chlamydospore production common.

These cultures showed basically the same progression as those on agar, with robust hyphae initially, followed by the production of conidia, attenuation of hyphae, autolysis, and chlamydospore production, with, after a few days, a final stage of a small proportion of hyphae maintaining irregularly staining cytoplasm, a large proportion of autolysed hyphae, and some inhibited conidia and chlamydospores.

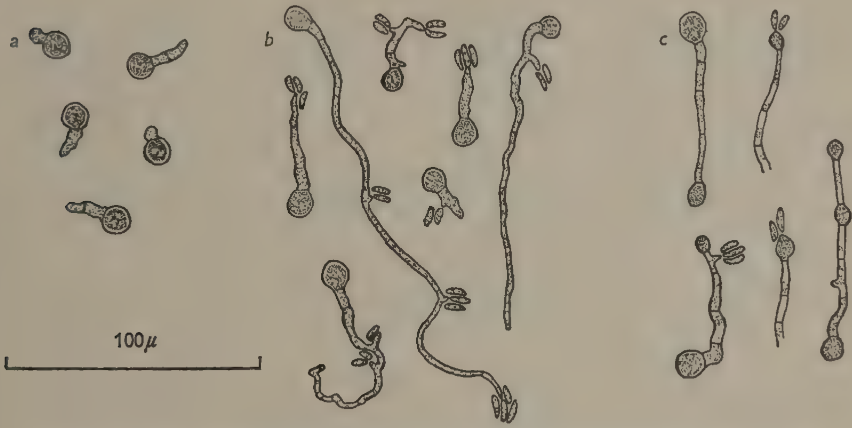
Drops of staled liquid from 17-day cultures were put on slides in a humid chamber and inoculated with fresh conidia. After 17 hr., 28 % of the conidia present were germinated, the resulting hyphae being more vacuolate and irregular in staining than those produced from conidia in the unstaled control, in which 59 % of the conidia were germinated. After 41 hr., the fungal structures in the two series were indistinguishable, both showing evenly-staining branched hyphae with robust apices, and with conidial production just beginning. No autolysis or chlamydospore production was observed in either solution at this stage. Thus the same solution that in the original staled culture had prevented germination, subsequently, on removal from the culture, allowed and supported good growth and development. In fact the ability of the solution to maintain growth appeared to have increased with time of standing. This observation was supported by others made from drops of staled culture liquid removed from a 20-day culture and containing inhibited conidia and chlamydospores and moribund hyphae. The drops were incubated on slides in a humid chamber without further inoculation. After 24 hr., the indigenous conidia and chlamydospores had germinated and the hyphae had grown on to produce branched mycelium with robust, evenly-staining apices.

These results indicate that at least some substance involved in staling is labile, and disappears or becomes ineffective under certain conditions. That these conditions may be satisfied in normal culture was shown by removing the mass of mycelium from a 20-day culture which had been apparently static for 2 weeks. After incubation of the solution for 24 hr., colonies up to 4 mm. diam. were present and were found by microscopical examination to have robust, evenly-staining hyphae. The colonies increased in size to *c.* 7–8 mm. diam. at 3 days, when the total amount of mycelium appeared slightly less than that taken out initially. The culture at this stage showed normal staling and no further obvious growth occurred.

Effect of mild heat on staled agar

Some of the observations recorded here suggest that the toxicity of a staled culture may normally be maintained by a low level of activity in the living hyphae present. It was found that reliably to kill the fungus in a 14-day Petri dish culture on agar by dry heat at 60°, a minimum exposure time of 2 hr. was necessary. In cultures exposed to this temperature for only

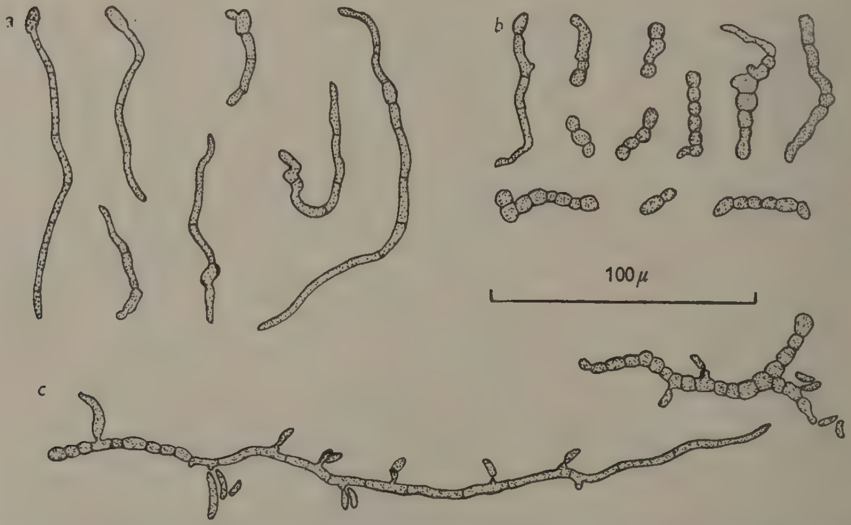
1½ hr., some plates showed complete killing while in others the hyphae and conidia were made inviable but chlamydospores survived in some areas of the plates. Plates treated for 1½ hr. were observed after further incubation under normal growth conditions. After 24 hr., the chlamydospores in the areas of survival had produced short germ-tubes (Text-fig. 1 *a*). At 44 hr. a little further growth had taken place but many of the germ-tubes were still unbranched. In those sporelings on the agar surface, the apices of nearly all of the laterals and of most of the main axes were abstricting microconidia (Text-fig. 1 *b*). Apices beneath the surface were non-reproductive. Little further growth in length occurred within the areas of germinated chlamydospores, and on the third day few vegetative apices remained, most bearing terminal chlamydospores (Text-fig. 1 *c*), and autolysis of hyphae had begun. This account applies to the developments within the areas of chlamydospore survival. Chlamydospores germinating on the margins of these areas gave germ-tubes that showed continued extension growth and normal branching. This growth entered the areas where complete killing by heat had occurred and produced juvenile hyphae, broad and straight at the margin, with the normal sequence of development as found on fresh agar.



Text-fig. 1. Germination and behaviour of indigenous chlamydospores in heated staled agar, after (*a*) 24 hr., (*b*) 44 hr., (*c*) 76 hr.

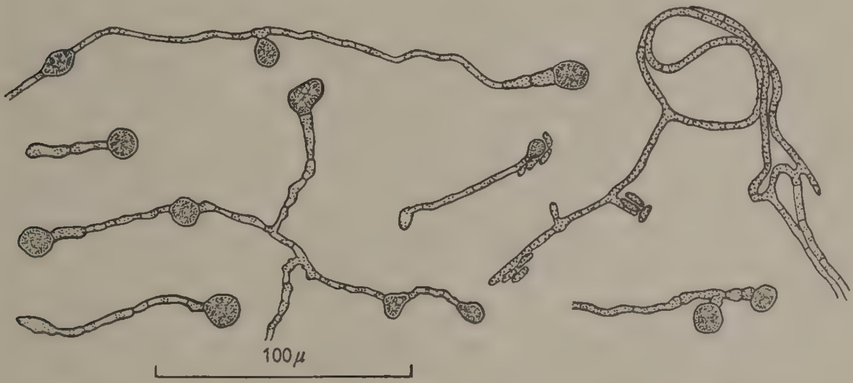
The developments illustrated here cannot be explained as a direct effect of the heat treatment of the fungus, since normal development follows in areas where no indigenous growth is present. The obvious explanation is that the heat kills the active hyphae present, allowing the disappearance of inhibitory substances and consequent germination of those chlamydospores that have survived. In some areas this inoculum is very dense and the renewed activity rapidly builds up a level of staling that inhibits extension growth, stimulates conidial production and then chlamydospore formation. This behaviour corresponds with that found in seeded plates, the inoculum being very dense. In areas where no indigenous inoculum survived the toxicity disappears, invasion takes place from the margins, and the type of development found on centrally inoculated plates is seen.

These conclusions were confirmed by heating unstaled and 17-day staled plates to 60° for 2 hr., cooling, surface-inoculating with dry smears of conidia, and incubating. In the unstaled control after 17 hr., the conidia had germinated normally with short undistorted germ-tubes (Text-fig. 2 *a*). In the staled culture the added conidia, which could readily be differentiated diffractively from the killed indigenous conidia, had germinated, but while a few spores, particularly some of those on the margins of the inoculum, showed undistorted germ tubes, most had produced a torulose, highly septate growth (Text-fig. 2 *b*). At 22 hr. the control showed good growth and, at the centre of the inoculated areas, microconidial production, although some of the later-germinating conidia here exhibited a mild torulose development. In the staled culture, conidial production from the torulose germ-tubes was common in the mass of the inoculum, but at the margins normal non-sporulating hyphae were growing out into the uninoculated areas (Text-fig. 2 *c*). The inhibition had thus disappeared from the uninoculated areas in the staled culture, while in the previously unstaled controls the germinating conidia had built up their own staling in the more densely inoculated regions. At 41 hr., colonies in the two series of plates appeared almost identical, both having broad, straight juvenile hyphae at the margins and the maturation sequence being normal. Plates given the same heat treatment, but incubated 24 hr. before re-inoculation, showed normal germination and development of added conidia in both the staled and unstaled series.

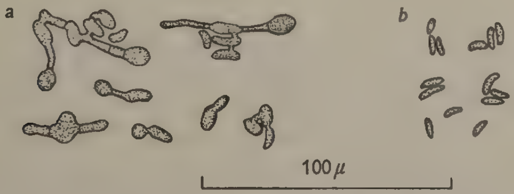


Text-fig. 2. Inhibition in a killed, staled agar culture. (*a*) Germination of conidia added to heated unstaled agar (17 hr.). (*b*) Germination of conidia added to heated staled agar (17 hr.). (*c*) Developments from conidia in heated staled agar (22 hr.).

A mild heat treatment, then, may kill the fungus while allowing some at least of the staling effect to remain in the agar. This is qualitatively detectable by immediate inoculation with conidia, but on standing for 1 day the toxicity becomes undetectable by this means. With decreasing toxicity in the previously staled agar, one finds firstly activity of the inhibited fungus giving chlamydospore-like structures, then conidium formation, and finally juvenile growth, the reverse sequence to that obtaining with the normal accumulation of staling substances in a growing culture.



Text-fig. 3. Developments after 5 days from conidia on the surface of Cellophane over a staled agar culture.



Text-fig. 4. Effect of Cellophane lying over spores on staled agar, after 5 days. (*a*) Germination of conidia and chlamydospores in areas beneath Cellophane; (*b*) conidia not covered by Cellophane.

EFFECT OF CELLOPHANE ON STALING

Boiled, washed and autoclaved Cellophane was put on the surface of 14-day staled agar cultures, and dry micronidia smeared on the Cellophane. After 19 hr. incubation, a number of the added conidia had produced short germ-tubes, and at 48 hr. more than 70 % of the conidia had germinated, the germ-tubes averaging about 100 µ in length. At 5 days a moderate amount of faintly staining and attenuate mycelium was present, though juvenile apices were rare, most hyphae ending in chlamydospores or producing microconidia (Text-fig. 3). Cellophane put on staled agar and incubated 24 hr. before inoculation gave identical developments. It is clear that some inhibitory effect is transmitted through the Cellophane, but the level of fungal activity was considerably greater than that found on the surface of cultures without Cellophane. The

Cellophane has an ameliorating effect on the action of the staling substances. This effect was also observed in the staled culture itself underneath the Cellophane. Microconidia and chlamydospores lying on the surface of the staled agar in areas covered by washed sterilized Cellophane had germinated after 3 days to produce short germ-tubes, which commonly fused with those from adjacent spores. No extensive mycelium was produced. Germination was not found in the same plates in areas not covered by Cellophane (Text-fig. 4).

TESTS FOR VOLATILITY OF STALING SUBSTANCES

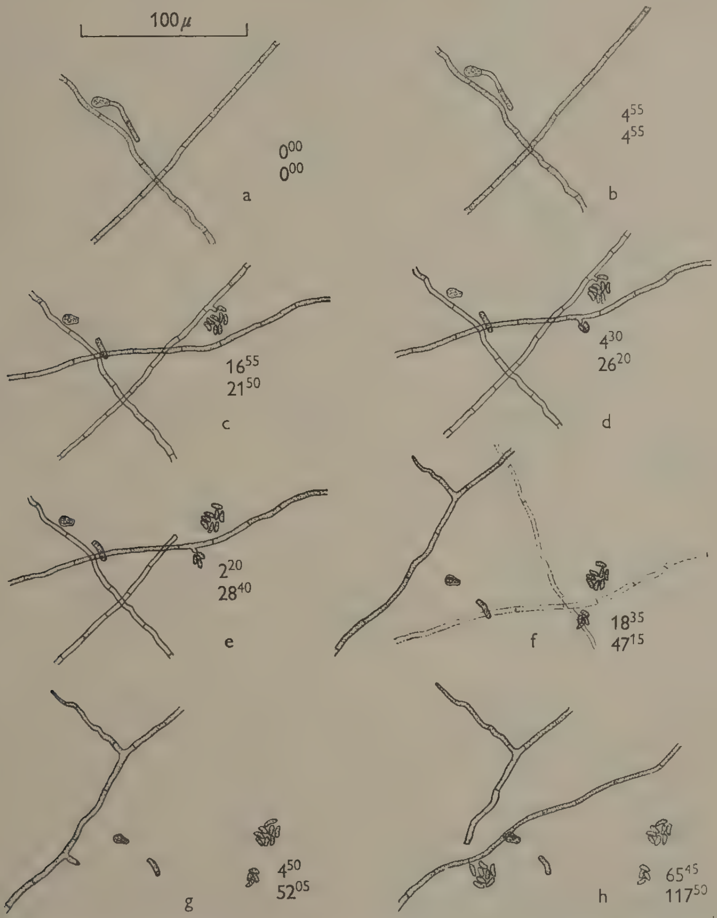
A probable explanation for the disappearance of the staling effect with time was volatility of the substances. The occasional aerial hyphae that develop on the agar used here produce microconidia and chlamydospores, and this could be caused by translocation of the stimulus in the hyphae, by its production in situ in the hyphae, or by a volatile stimulus. It is recorded (Brown, 1922) that a volatile product of microbial growth may inhibit germination of conidia of a number of fungi, and it has further been suggested (Cochrane, 1958) that the substance causing self-inhibition of uredospores of rust fungi is of a volatile nature.

Slabs of sterile distilled water agar (1 mm. thick) and of nutrient agar were put in the lids of Petri dishes containing 17-day staled cultures on nutrient agar. The slabs were inoculated with conidia of *F. oxysporum* at intervals from the start to the fifth day. All showed normal germination, hyphal growth and colony formation. No evidence was obtained of volatility of the substances.

SECONDARY ACTIVITY IN STALED CULTURES

The evidence that there is a loss of the staling effect with time, and that some fungal developments may occur after this loss, suggested that some of the developments observed may be normal in old, apparently static, staled cultures. Indeed, evidence that hyphal growth and conidium and chlamydospore formation do occur in staled seeded plates has already been presented. In order to obtain more convincing data, some detailed examinations of 14–17-day staled agar cultures were made with medium magnification of the microscope, and various structures indicating secondary activity were recorded. The observations recorded in the explanation of Pl. 27, figs. 1–9, together strongly indicate continued activity in the staled culture, the apparently static condition being a result of a gross view of a dynamic situation in which growth and autolysis are balanced at a given level for any particular set of environmental conditions.

Further evidence for this sort of dynamic situation was obtained from a series of observations made on living, untreated 17-day staled agar cultures. Camera lucida drawings were made of selected small areas of culture, containing characteristic configurations of chlamydospores to act as markers. During periodic observations over several days some of these areas were lost, probably owing to the pattern having changed beyond reliable recognition, but in those that remained identifiable some changes did occur. These involved growth and disappearance of hyphae with the persistence of chlamydospores and some hyphal segments, production of microconidia and chlamydospores, and chlamydospore germination. Sample stages from a typical sequence are shown in Text-fig. 5. The life of an individual hypha from its inception, through conidial production, to autolysis and disappearance may be less than 24 hr. Burges (1958) has calculated that the life in soil of some individual pieces of mycelium is less than 1 day.



Text-fig. 5. Sequence of developments in an area of an untreated staled agar culture. The upper numerals in each drawing indicate the interval in hours and minutes from the previous observation, the lower numerals the interval from the start of observations.

HYPHAL DENSITY AFTER A PERIOD OF LOWERED ACTIVITY

If, as suggested, the staling substances are produced by hyphal activity and attenuated with time, then it would be expected that under some circumstances at least, an imposed restriction of hyphal activity in a staled culture should be followed by a decrease in the level of toxicity, subsequently permitting a higher level of activity after removal of the restriction. Staled plates (15 day) were put at 4° and others at 37° for 6 days, and then returned to normal incubation conditions for 24 hr. Hyphal density in these plates was then compared with that in control plates, by counting presence or absence of stainable hyphae in 500 microquadrats of side 2·5 μ (Table 2). Hyphae were almost twice as frequent in the heat-treated plates as in the controls, and more than four times as frequent in the plates having received the cold treatment.

Table 2. *Effect of reduction in activity on subsequent hyphal frequency in staled agar*

	No. 6·25 μ^2 quadrats/500 with living hyphae	Percentage frequency
Before treatment	77	15·4
After 37°	171	34·2
After 4°	363	73·0
Untreated control	87	17·4

Table 3. *Staling in fungi other than* Fusarium oxysporum

	Lysed hyphae	Living hyphae	Secondary growth	Asexual spores	Chlamydospores	Persistent hyphal segments
Absidia orchidis	+	+	+	+	−	−
Armillaria mellea	Few	Much	−	−	−	−
Aspergillus oryzae	+	+	+	+	−	+
A. niger	+	+	+	+	−	+
A. nidulans	+	+	+	+	−	+
Botrytis cinerea	+	+	+	+	−	+
Chaetomium globosum	+	+	+	−	−	+
Helminthosporium sativum	+	+	+	+	−	+
Merulius lacrymans	Few	Much	−	−	−	−
Mucor spinosus	+	+	+	+	+	−
Neurospora crassa	+	+	+	+	−	+
Penicillium digitatum	+	+	+	+	−	+
P. notatum	+	+	+	+	−	+
Pestalotiopsis gracilis	+	+	+	+ (2)	+	+
Pyronema omphalodes	+	+	+	−	−	+
Pythium debaryanum	+	+	+	*	+	−
Sordaria fimicola	+	+	+	−	−	+
Stemphylium sp.	+	+	+	−	−	+
Trichoderma viride	+	+	+	+	+	+
Trichothecium roseum	+	+	+	+	−	−
Xylaria polymorpha	Few	Much	−	−	−	−
Zygorhynchus moelleri	+	+	+	*	+	−

* *P. debaryanum* produced oospores and resting sporangia, *Z. moelleri* produced zygospores and sporangiospores.

SECONDARY ACTIVITY IN OTHER FUNGI

Agar cultures of twenty-two other fungi were incubated for 30 days and examined. Observations were made for the presence of lysed hyphae and of stainable hyphae. Particular attention was paid to evidence of a dynamic situation as shown by secondary activity having taken place after initial staling. Notes were also made on structures that might assist in maintaining viability after lysis of hyphae. The results are summarized in Table 3.

Nineteen of the fungi (including species belonging to the Phycomycetes, Ascomycetes and Fungi Imperfecti) showed in their cultures a situation similar to that found in *F. oxysporum*, with much hyphal lysis, a low density of living hyphae, and probable recent secondary activity. All nineteen had some form of resting structure in the cultures. Some had more than one sort, like *Pestalotiopsis* with two sorts of conidium, chlamydospores, and persistent hyphal segments (and probably also the acervular stromata); others had only one sort, like *Absidia* with sporangiospores, and *Sordaria* and *Pyronema* with persistent hyphal segments only.

Three of the fungi, *Armillaria*, *Merulius* and *Xylaria*, showed no resistant resting structures and little lysis. In these an intact hyphal network was maintained in the old cultures, and there was no evidence of its having been secondarily formed after lysis. The hyphae retained the juvenile form. These three fungi have in common, and in this differ from the other twenty observed at this stage, the possession in their life cycles of bulky sporophores, the production of which requires a substantial reserve of foodstuffs supplied through an anastomosing hyphal system (Buller, 1931).

Table 4. *Staling in fungi having bulky sporophores*

	Lysis	Anastomosing hyphal network
Armillaria mellea	Rare	Complete and dense
Merulius lacrymans	Some	Complete and dense
Xylaria polymorpha	Rare	Complete and dense
Fomes annosus	Moderate	Complete
F. igniarius	Moderate	Complete
Ganoderma applanatum	Moderate	Complete
Lenzites sepiaria	Some	Complete and dense
Phaeobulgaria inquinans	Moderate	Complete and dense
Stereum sanguinolentum	Moderate	Complete and dense
Trametes serialis	Moderate	Complete
Pellicularia subcoronata	Much	None

This correlation was carried further by examining 30-day staled cultures of one other ascomycete and six other basidiomycetes that produce massive sporophores (Table 4). *Pellicularia subcoronata*, which was added to the series as a basidiomycete not having a bulky sporophore, behaved in the same way as the other stylosporic species, showing extensive lysis, a little viable mycelium which did not maintain a complete network, and producing as resistant stages its basidiospores and persistent hyphal segments. There was a clear indication of secondary activity in the cultures of this species. The other seven fungi showed no resting stages in these cultures, and, although the amount of lysis varied from little to moderate, all maintained a complete anastomosing system of stainable juvenile hyphae with no evidence of secondary development. Since observations have so far been confined to pure cultures of single fungi, it is not known whether the ability of these fungi to maintain the juvenile hyphal system resides in a low production of staling substances or in a high tolerance to their presence.

DISCUSSION

The development of autotoxicity has commonly been referred to in the past as staling, and until the process is better understood, it seems convenient to continue this usage. The demonstrated transience of the staling effect in culture media suggests that staling substances may have properties in common with the labile hormones of fungi (Cantino & Turian, 1959). The evidence indicates that the transience of the substances, which is best shown by removal or inactivation of the mycelium, is also found in the presence of the mycelium, and that the apparently static condition of the staled culture is an artefact produced by a gross view of a system in which loss of staling and hyphal activity, producing staling, are dynamically balanced. The situation is in some ways comparable with that described by Watt (1947) for green plants.

It is proposed here that the staling substances not only have an inhibitory effect on the fungus but also have positive morphogenetic effects. The hypothesis is that, as any area of the fungus gets older, the concentration of staling substances increases and affects qualitatively the growing hyphal apex. First, there is a change from a broad juvenile hypha to a narrow hypha, concurrently with the change from centrifugally orientated growth to a meandering growth. The narrower hypha is a reflexion of the smaller amount of wall material synthesized per unit growth in length; the lack of orientation after the formation of closed areas in the culture comes about from the loss in the area of the concentration gradient of staling substances which determines orientation (Graves, 1916). At higher concentrations of the staling substances, normal elongation growth is inhibited but the apex abstricts conidia, while at still higher concentrations swelling of the apex results in chlamydospore formation. At these levels spore germination is inhibited. At the highest concentration, complete cessation of activity and onset of autolysis follow, although individual hyphae may differ in their resistance to autolysis. At this point, of course, production of the staling substances ceases and their concentration begins to decrease. With loss of the substances from the medium, by natural lability or by diffusion outwards, renewed activity of inhibited hyphal segments and spores occurs, with the production of torulose hyphae, chlamydospores, conidia, and normal vegetative hyphae in that order, with increasing liberation of the apex. It is, of course, possible that each of the different effects described—autolysis, inhibition of growth, and the separate morphological developments—depends on a specific agent, but there is nothing in the observations recorded to suggest that a single substance or type of substance is not responsible.

It is significant that the morphogenetic and staling factor accumulates not in the fungus but in its environment. The morphology of the fungus then is governed not primarily by its own age and past history, but by the history of its environment. Staling effects of the sort described for *F. oxysporum* were found in pure cultures of a number of other filamentous fungi. It is known that fungi can have morphogenetic effects on other fungi. It consequently seems probable that fungal apices may react in the same way to a number of naturally occurring chemical substances, particularly when, as here, the effect is one of shifting the apex away from its normal juvenile condition. However, in all the fungi showing the effect there was evidence of the lability of the agent, and a group of substances having at least this property in common may be postulated.

Pratt in her classical work on cultural staling (1924*a*, *b*) concluded that formation of bicarbonates in the medium could account for the phenomena there recorded. No chemical analysis of the staled medium has been made in the present investigation, but under the complex conditions of a growing culture it is not impossible that bicarbonates might have a limited stability, and the observations here are in this respect not inconsistent with the effect recorded by Pratt. However, other workers, including Pratt, have found the staling of liquid media to be of a form sufficiently stable to be tested by spore germination. There are several possible reasons for this difference. Pratt (1924*a*) lists at least three different sorts of staling of varying stability. Also the level of nutrients in the medium may affect both the level of staling substances accumulating, and the tolerance of the fungus in different stages to given levels of the substances. Thus in a more concentrated medium a higher level of labile staling substances may remain stable longer, or the conditions of the medium may favour their stability, or the type of staling may be qualitatively different. Nevertheless, Pratt (1924*b*) indicates that a dynamic balance between growth and autolysis of hyphae does occur in the richer cultural media but that at that stage the capacity of the medium to support spore germination is unimpaired. It seems that this last point was determined by removing the liquid from the culture for testing, and it is not stated whether spores produced in the culture were at this stage able to germinate. There remains therefore the possibility that lability of the staling substances occurred under those conditions also, and that the distinction between the two types of staling—that assessed by autolysis and that assessed by capacity to support spore germination—may be less clear than appeared.

Whether the type of staling studied in this work and that occurring under more normal laboratory conditions differ qualitatively or only quantitatively, there occur in nature habitats similarly low in nutrient status and it is possible that the phenomena described here may be relevant to the biology of fungi there.

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EXPLANATION OF PLATE 27

Structures in untreated, staled agar culture. All ×940.

Fig. 1. Secondarily produced hyphal coil with microconidia.
Fig. 2. Recently produced chlamydospore.
Fig. 3. Chlamydospores and persistent hyphal segment after autolysis of parent hypha.
Fig. 4. Lysis of chlamydospore and persistent hyphal segment.
Fig. 5. Persistent hyphal segment with conidiophore.
Fig. 6. Germinating chlamydospore.
Fig. 7. Autolysis of conidia.
Fig. 8. Germination of conidium.
Fig. 9. Germination of persistent hyphal segment.

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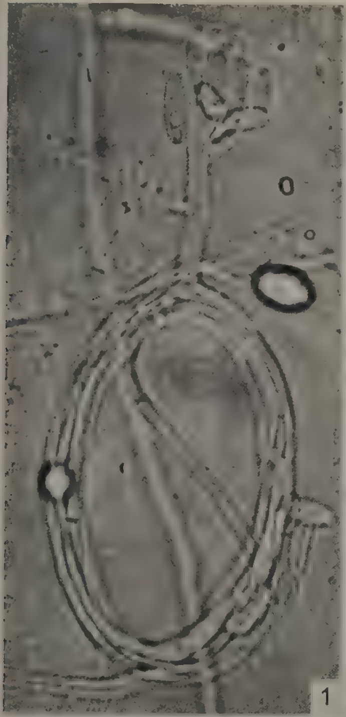
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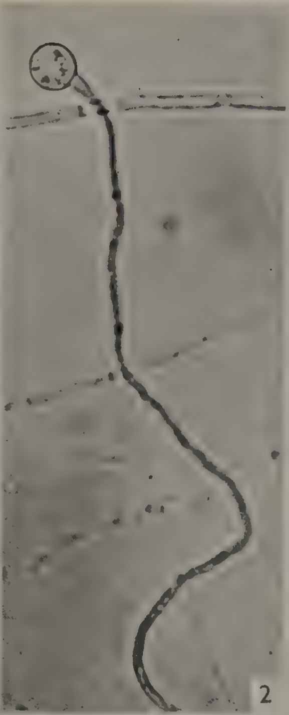
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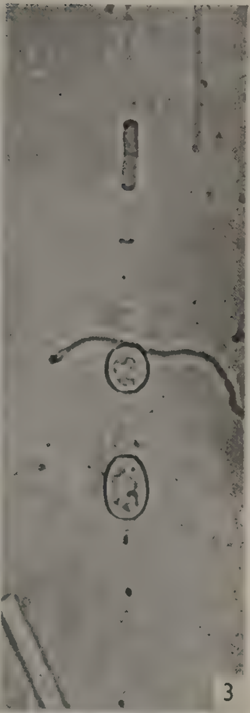
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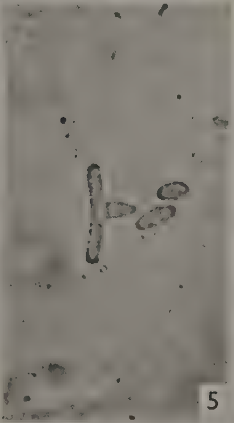
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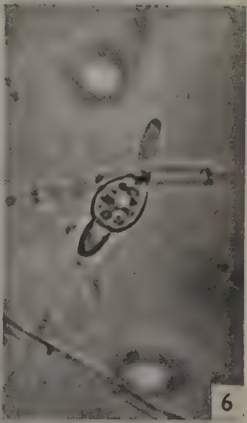
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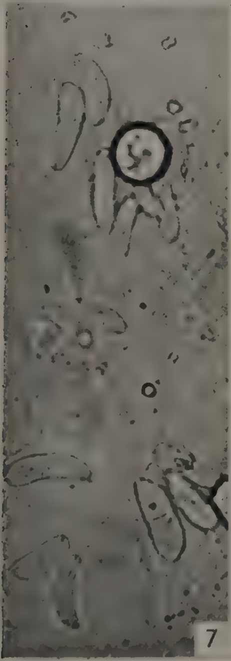
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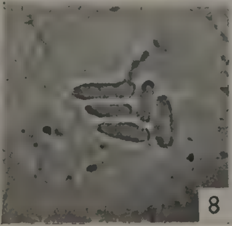
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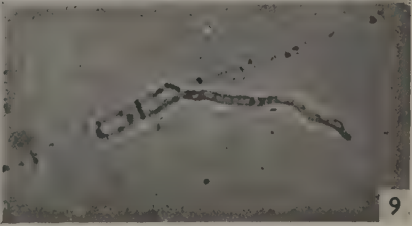
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FRUIT-SPOT ('SPECKLE') OF JAMAICAN BANANAS CAUSED BY *DEIGHTONIELLA TORULOSA* (SYD.) ELLIS

III. SPORE FORMATION, LIBERATION AND DISPERSAL

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(With Plate 28 and 6 Text-figures)

The air in two Jamaican banana plantations was sampled during the period 19 July–5 October 1960 at 3 m. above ground with a Hirst automatic volumetric spore trap. Each day's slide was scanned and conidia of *Deightoniella torulosa* were counted at positions representing 2-hourly intervals. In both plantations there was a clearly defined diurnal periodicity, the peak concentration of spores/m.^{3} of air normally occurring near 08.00 hr. and then decreasing to a low level between 12.00 and 24.00 hr. each day. The period of most rapid liberation (06.00–10.00 hr.) corresponds with that of rapidly increasing temperature and decreasing R.H. This may be due to a previously undescribed active discharge mechanism operating under conditions of decreasing vapour pressure.

In plantation A, which was surface irrigated, daily mean concentration varied between 0 and 58 spores/m.^{3} when the previous 2–12 days were rainless. After 0·55 and 2·55 in. rain, concentrations of 377 and 536 spores/m.^{3}, respectively, were recorded: the highest 2-hourly estimate recorded was 5280 spores/m.^{3}. In plantation B, where under-tree irrigation was practised, successive irrigation periods (each of 48 hr.) were associated with considerable increases in spore concentration; rainfall resulted in similar large increases. The highest daily mean concentration recorded was 7115 spores/m.^{3}, while the highest 2-hourly estimate was 72,335 spores/m.^{3} at 08.00 hr. on 8 September—approximately 12 hr. after cessation of irrigation. As the plantation dried out after irrigation or rainfall, spore concentration decreased rapidly within 2–3 days.

Both rainfall and irrigation spray resulted in heavy sporulation of *D. torulosa* on banana-leaf 'trash'; this helps to explain the increased atmospheric spore concentrations referred to above. There was a marked increase in incidence of fruit-spot during under-tree irrigation periods and when rainfall caused the plantation R.H. to remain high for more than 12 hr. during the day. Incidence of fruit-spot in plantation B was higher than in A; this was related partly to the greater numbers of dead banana leaves in the former, and partly to the favourable effects of under-tree irrigation on sporulation, spore germination and infection.

Recent studies (Meredith, 1960, 1961 *a*, *b*) have shown that a fruit-spot disease ('speckle', 'swamp-spot') of Jamaican 'Lacatan' bananas is caused by *Deightoniella torulosa* (Syd.) Ellis. The fungus has been reported to cause a 'black-spot' disease of banana leaves (Ashby, 1913; Stahel, 1934; Meredith, 1960 *b*) and a blackening of the tip of banana fruits ('black-tip', Ogilvie, 1928; Wardlaw, 1932; Meredith, 1960 *b*). *D. torulosa* is a common saprophyte in Jamaican plantations, sporulating on dead or dying leaves and bracts, and on withering perianths, staminodes and styles of the banana plant.

The introduction by Hirst (1952) of an automatic volumetric spore trap has enabled mycologists to collect detailed information about various components of the air spora at any locality (Hirst, 1953, 1959; Hirst, Storey, Ward & Wilcox, 1955; Gregory & Hirst, 1957; Gregory & Sreeramulu, 1958; Gregory & Stedman, 1958; Sreeramulu, 1959). In the present investigation a similar trap was operated successively in two banana plantations. The primary object was to study spore formation, liberation and dispersal in *D. torulosa* in relation to time of day, weather, plantation conditions and outbreaks of fruit-spot. Information collected simultaneously about the dispersal of other fungi, saprophytic or parasitic on bananas, will be published elsewhere.

MATERIALS AND METHODS

Plantation conditions

The plantations selected for study are situated on the plains of St Catherine (elevation not exceeding 200 ft.) where extensive sugar-cane and banana plantations are established. Plants were predominantly of the 'Lacatan' variety with a few scattered 'Robusta' plants.

Plantation A (*Cow Park*). This was third ratoon crop (600 plants/acre) and many plants were bearing fruit, some of which were ready for harvest at the commencement of trapping. The field was irrigated at fortnightly intervals by admitting water into shallow trenches between the plant rows (surface irrigation). Aerial spraying with oil was carried out at 3-week intervals at the rate of 0·5–0·75 gal. oil/acre. This treatment has resulted in excellent control of leaf-spot disease (*Mycosphaerella musicola*) in this region of Jamaica. Almost every plant was bearing one or more collapsed, dead or dying leaves on which *D. torulosa* sporulated at certain times. Isolated instances of 'black-spot' disease were noted but 'black-tip' was not observed. An account of the incidence of fruit-spot will be given later in the text.

Plantation B (*Cedar Grove*). In this plantation, under-tree irrigation was carried out at various intervals, depending on the distribution of rainfall. Here, water was mechanically pumped along portable metal pipes laid between plant rows. The water was ejected from revolving nozzles situated at *c.* 35 ft. intervals along the pipes. Since the water is sprayed several feet into the air, bunches of fruit and upright and collapsed leaves are thoroughly wetted; pumping is normally continued for about 48 hr. The crop was a 'plant' crop (909 plants/acre) and, as in plantation A, bunches of fruit at all stages of maturity were present. However, there was considerably more banana-leaf 'trash' in plantation B. Other conditions were as already noted for plantation A.

Trapping and scanning procedure

A Hirst trap was set up inside the plantation so that its orifice was 3 m. above ground (Pl. 28, fig. 1). In plantation A, sampling at the rate of 14·4 m.^{3}/24 hr. was commenced on 19 July 1960, and continued until 28 August 1960. The sampling period in plantation B was 1 September–

5 October 1960. The slides, prepared as described by Hirst (1953), were changed daily at 09.00 hr. E.S.T. After exposure, slides were mounted for examination in 'Solvar'-lactophenol mixture (Hirst, 1953) and scanned under a high-power objective. Counts were made on cross traverses 40 μ wide and 4 mm. apart, representing 2-hourly intervals, starting at 10.00 hr. each day. The number of conidia of *D. torulosa* counted was then converted into an estimated number/m.^{3} of air.

Conidia formed under natural conditions on dead leaves, bracts or flower parts are relatively large ($35–70 \times 13–25\,\mu$) and characteristic (Meredith, 1961*a*). Representative conidia, trapped in the above described manner, are shown in Pl. 28, fig. 2.

Hirst (1953) points out that the efficiency of this type of trap is always less than 100 %, varying according to wind speed and particle size. In the current investigation, recording anemometers were not available and it was not possible to apply corrections for variation in efficiency. Thus all spore numbers quoted are underestimates of the true concentration.

Meteorological data

Daily records of temperature and R.H. were obtained by means of two thermohygrographs mounted on the trap-supporting stand. Two rain gauges were placed in a clearing close to the trap.

SPORE FORMATION

Before presenting the results of trapping, it is desirable to describe some observations on sporulation of *D. torulosa*. It has been noted already that the fungus is a common saprophyte on dead banana leaves (Meredith, 1960*a*, *b*). It appears to be a relatively late colonist, being abundant on leaves that have dried out considerably and become colonized by fungi such as *Gloeosporium musarum* and *Nigrospora* (?) *oryzae*. The presence of *D. torulosa* is clearly indicated by dark coloured areas on the petiole and midrib; the upper regions of the leaf sheath, and the broad, tattered lamina are not normally extensively invaded. In dry weather, when the petiole and midrib have a very low moisture content, the dark areas colonized by *D. torulosa* are found to bear conidiophores only. The structure and development of conidia and conidiophores have been described in detail by Wardlaw (1932). After the single terminal conidium is liberated, the conidiophore may proliferate in one of several ways to form a new conidiophore head capable of producing another conidium. This process may be repeated several times under favourable environmental conditions, so that the conidiophore becomes much 'elbowed' (Pl. 28, fig. 3).

Observations in the plantations revealed the presence of only small numbers of viable conidia on dead leaves during dry weather. When there was rainfall sufficient to wet the leaves, particularly if it fell during late afternoon or early evening, abundant sporulation occurred overnight. The effect of external humidity on sporulation was investigated further in the laboratory. Sections of petiole or midrib naturally colonized by *D. torulosa* were air-dried and then placed in a strong air current in order to remove any conidia. The absence of conidia was confirmed by direct microscopical examination. Some sections were stored in desiccators having atmospheres of known R.H., while others were wetted and incubated in a damp chamber. Samples were removed at various intervals, and the development of new conidia was recorded. After 12 hr. incubation, sporulation was abundant on petiole sections which were wetted or stored at 100 % R.H. There was slight conidium development at 90–95 % R.H. but none at humidities lower than 90 % R.H.

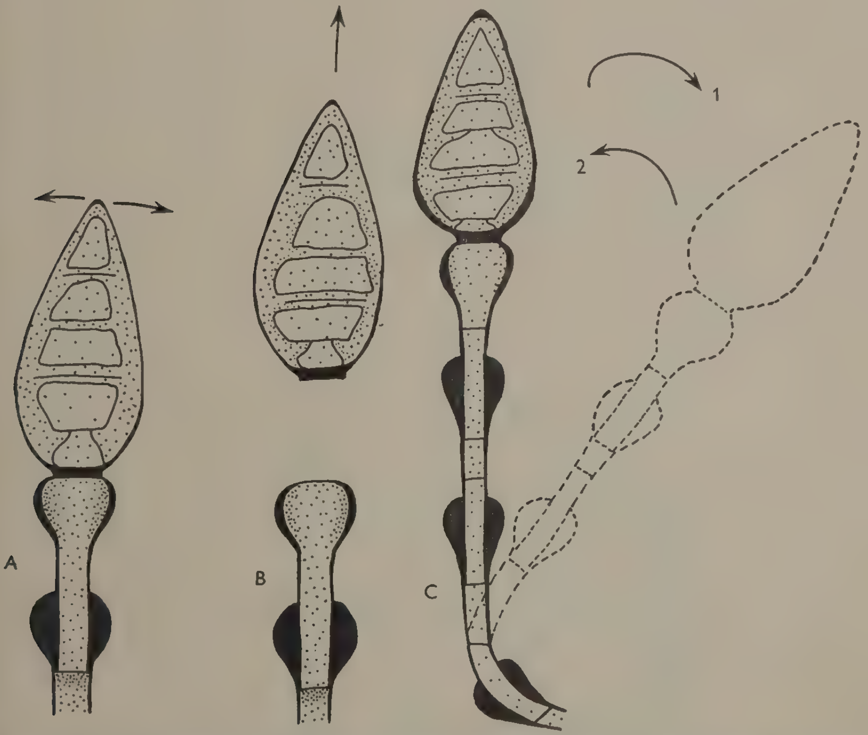
SPORE LIBERATION

Ashby (1913) noted that conidia readily fall away from the rounded conidiophore heads and that they are rarely attached when infected banana leaf tissue is examined in the laboratory. The author is unaware of any other references to spore liberation in *D. torulosa* and thus some more detailed observations were made in this investigation.

When wet, dead banana leaves were collected early in the morning, stored in air-tight bags and examined while still wet, it was found that the majority of conidia were attached to the conidiophores. However, if the leaves dried out to some extent between the times of collection and examination, most conidia had been liberated. The process of liberation was observed on thin strips taken from the surface of a banana-leaf petiole colonized by *D. torulosa*. The strips were wetted and then incubated for 24 hr. inside damp Petri dishes, in order to induce abundant spore formation. They were then transferred quickly to a glass slide and examined under a low-power objective (× 190) using incident light. Violent spore discharge commenced immediately after transfer and more than 90 % of the spores had been liberated after 1 min. Just before discharge occurs, the terminal conidium undergoes several side to side movements as though pivoted on the conidiophore head (Text-fig. 1 A); simultaneously, several large vacuoles appear inside the conidium, one at the base usually appearing first. The conidium is then violently shot from the conidiophore to a distance commonly exceeding 10 mm. (Text-fig. 1 B). The speed of movement of the conidium is so great that it is not easy to trace its path of flight. At the time of discharge, the conidiophore undergoes rapid recoil and recovery. Sometimes before discharge occurs, the conidiophore displays hygroscopic movements. There is a slow bending over, followed by a sudden return to the original position (Text-fig. 1 C); this may be repeated several times, but to a decreasing extent. Such movements rarely result in liberation and, even when they do, the conidium is not projected more than a few microns.

It is possible that a sudden decrease in humidity is an important factor controlling liberation. The observations recorded above were made immediately after transferring material from R.H. 100 % to laboratory air (R.H. 78 %). Increased temperature, on transfer to the microscope stage, may also be of importance. It is not known whether light affects the process, as it does in the case of certain downy mildews (Yarwood, 1937).

It is not known whether the mechanism of spore discharge is similar to any reviewed by Ingold (1953). However, for the purposes of this paper, it is sufficient to note that there is an active discharge mechanism and so the conidia will stand a good chance of being projected through the non-turbulent layer at the surface of the spore-bearing substratum; further dispersal will then be determined by wind and eddy diffusion (Gregory, 1945). The relatively large conidia probably sediment quickly under the influence of gravity; Gregory (1951, 1952) has drawn attention to the greater efficiency of impaction of larger spores.



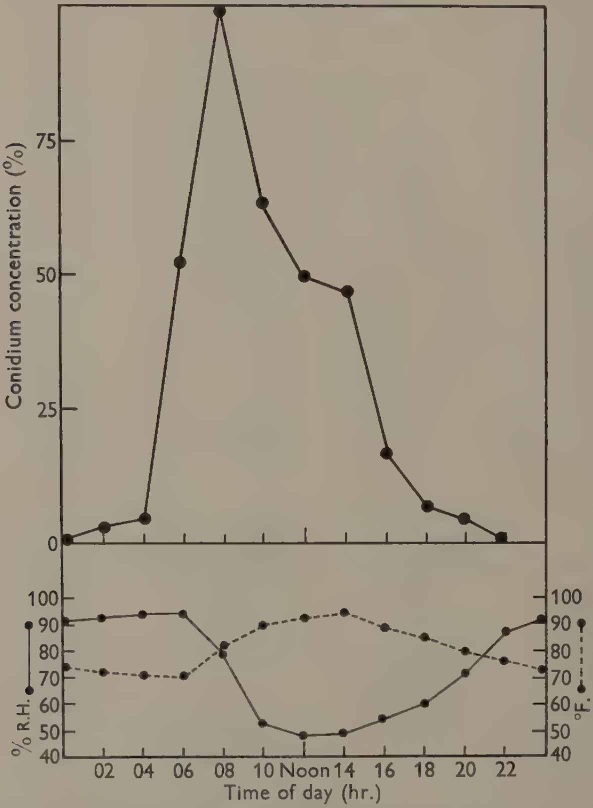
Text-fig. 1. A, Conidium of *D. torulosa* attached to swollen head of conidiophore just before discharge; B, just after discharge; C, hygroscopic movements of *D. torulosa* conidiophore: 1, slow bending movement; 2, rapid recovery to original position.

The operation of this active discharge mechanism may explain why Ashby (1913) rarely found conidia attached to conidiophores on diseased leaf material. If the material was collected and stored in an atmosphere of 80 % R.H. or less before examination, then liberation had probably occurred already. This has certainly happened in the author's experience, particularly when material was transported for some distance after collection. Liberation may be prevented if the leaf material is collected from the plantation at about 06.00 hr., before the R.H. falls below 90 %, and transferred immediately to a damp container.

DIURNAL PERIODICITY OF *DEIGHTONIELLA TORULOSA*

Plantation A

The mean diurnal periodicity curve (Text-fig. 2) was derived from the geometric means of the estimated numbers of conidia/m.³ of air at each even hour; data were plotted according to the method of Hirst (1953).

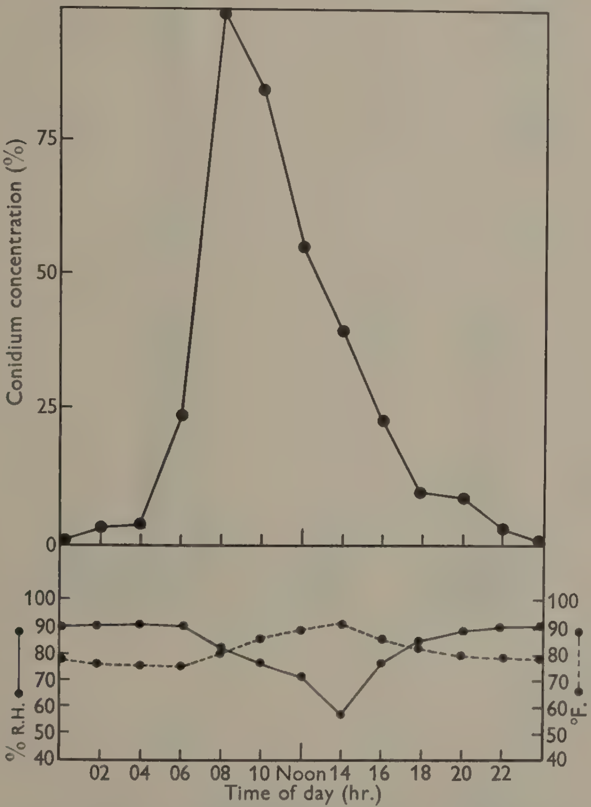


Text-fig. 2. Plantation A. *Above*, mean diurnal periodicity curve of conidium concentration, for *D. torulosa*, expressed as a percentage of the peak geometric mean concentration. *Below*, hourly values for temperature and R.H. during period 19 July–28 August.

There was a clearly defined diurnal periodicity, rapid liberation of conidia beginning shortly after 06.00 hr. and reaching a maximum near 08.00 hr. This period of rapid liberation corresponds quite closely with that of increasing temperature and decreasing R.H. After 10.00 hr. there was a rapid decrease in concentration and between 16.00 and 04.00 hr. it was uncommon to find more than 1–3 conidia per cross traverse.

Rainfall had a pronounced effect on the extent of daily fluctuations in spore numbers. During the dry periods 23–31 July and 3–11 August, the mean concentrations at 08.00 hr. were 56 and 124/m.³, respectively;

at 10.00 hr. they had decreased to 28 and 17/m.^{3}, respectively. When there had been more than a trace of rain on the previous day, particularly if it fell during late afternoon or early evening, the early morning peak concentration increased markedly. For example, on 21 July, 1, 12 and 15 August there were 0·92, 0·21, 0·63 and 2·55 in. rain, respectively. On the following days, maximum conidium concentrations of 3003, 759, 2673 and 5280/m.^{3}, respectively, were recorded between 06.00 and 08.00 hr.



Text-fig. 3. Plantation B. *Above*, mean diurnal periodicity curve of conidium concentration for *D. torulosa*. *Below*, hourly values for temperature and R.H. during period 1 September–5 October.

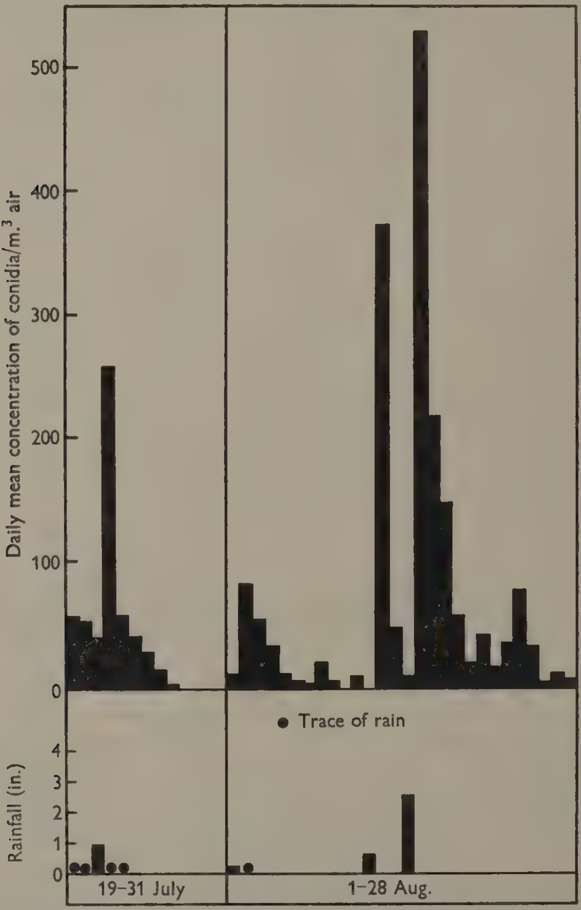
After rainfall there was a tendency for relatively high concentrations to persist until 12.00 or 14.00 hr. Later the numbers of conidia trapped decreased to levels comparable with those during dry weather.

Plantation B

Diurnal periodicity (Text-fig. 3) was similar to that in plantation A, peak concentrations occurring regularly at 08.00 hr. A more detailed analysis is made below.

SPORE CONCENTRATION AND DISEASE OUTBREAK

Daily mean concentrations of conidia were estimated by averaging the 2-hourly counts for the period 24.00–24.00 hr. each day.



Text-fig. 4. Plantation A. Daily mean concentration of *D. torulosa* conidia in relation to rainfall during period 19 July–28 August 1960.

Plantation A

Spore liberation and rainfall. In Text-fig. 4 the daily mean concentrations of conidia are related to total daily rainfall. Whenever there was measurable rainfall, there was a corresponding increase in concentration on the following day. The highest mean concentration recorded was 536/m.^{3} on 16 August. After each sudden increase, the concentration decreased to less than 100/m.^{3} within 1–3 days. On a total of 6 days, after hot, dry weather, no spores were trapped during the 24 hr. exposure period.

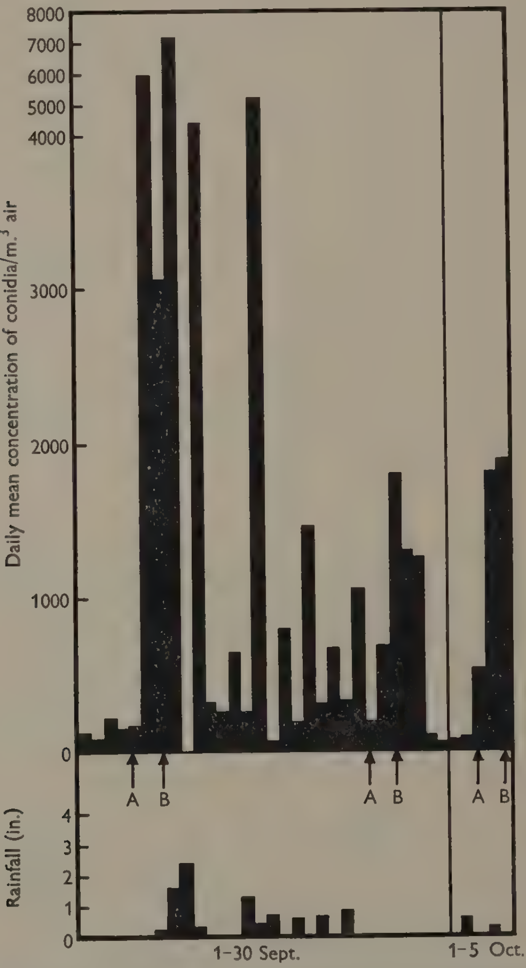
Disease outbreak. The incidence of fruit-spot was closely observed on seventy bunches of fruit that had just 'shot' at the commencement of trapping. This fruit was initially free from spotting, so the first appearance of disease could be accurately dated. On no day except 16 August did liberation of conidia result in the development of visible spots. Earlier studies (Meredith, 1961 *a*, *b*) have shown that, for infection to occur, R.H. 95 % or more must be maintained at the fruit surface for at least 6 hr.; percentage infection is highest when the fruit surface bears a film of liquid water. On 22 July, 2 and 13 August, maximum concentrations of conidia occurred at 08.00 hr. The rapid increase in temperature and decrease in R.H. which occurred between 08.00 and 12.00 hr. probably resulted in conditions becoming unfavourable for germination and infection. There was some slight development of fruit-spot following liberation on 16 August. This may be related to the fact that the morning was cloudy and the plantation dried out slowly, so that humidity conditions remained favourable for infection for at least 6 hr.

It is possible that isolated spots may develop during dry weather if conidia settle in dew droplets on the fruit surface, when several hours may elapse before the dew on heavily shaded fruit evaporates. However, in this investigation, it was not possible to relate such a low incidence of spotting to dry weather spore concentration.

Plantation B

Spore liberation, irrigation and rainfall (Text-fig. 5). There was no rainfall between 1 and 5 September, during which period the plantation had dried out since previous irrigation (20–22 August). During this period the daily mean concentration of conidia varied between 50 and 210/m.^{3}. Under-tree irrigation was commenced at 17.00 hr. on 5 September (arrow *A* in Text-fig. 5), but was discontinued between 07.00 and 10.00 hr. on 6 September, owing to pump failure. Pumping was then recontinued until 17.00 hr. on 7 September (arrow *B* in Text-fig. 5). On 6 September there was an increase in concentration to 5814/m.^{3}. Observations made on 5 September before irrigation started showed that conidia of *D. torulosa* were infrequent on dead banana leaves. However, at 07.00 hr. on 6 September, all dead leaves examined were bearing large numbers of fresh conidia, formed overnight. The continuous wetting of leaves by irrigation spray favoured sporulation, thus helping to account for an increased concentration of conidia on 6 September. Relatively high daily mean concentrations were obtained on 7 and 8 September (3058 and 7115/m.^{3}, respectively). On both dates at 07.00 hr., sporulation on dead leaves was abundant. Unfortunately the trap was inoperative at 08.00 hr. on 9 September, when a similar high concentration of conidia was anticipated, there being 1·48 in. rain on the previous day. On 10 September the mean concentration was 4477/m.^{3}. The 2·32 in. of rain recorded on the previous day resulted in dead leaves remaining wet throughout the night; all leaves examined at 07.00 hr. on 10 September were bearing large numbers of conidia.

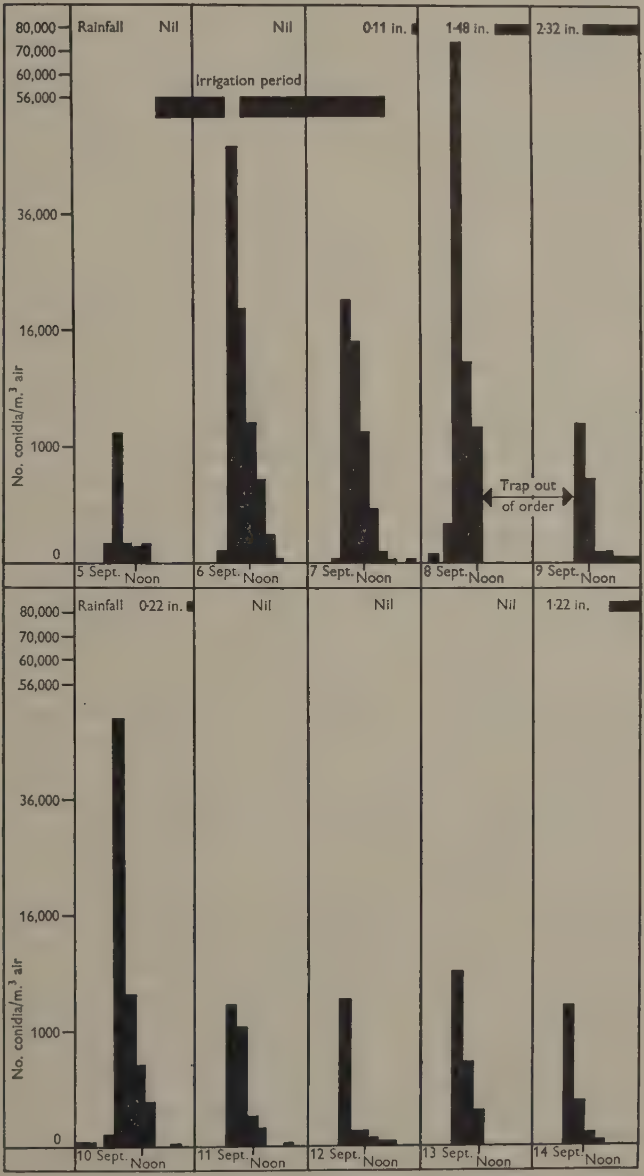
With the exception of an early morning rain shower (0·22 in.), 10 September was dry, with maximum temperature 93° F. at 14.00 hr. inside the plantation; many dead leaves dried out before nightfall. On 11 September the daily mean concentration of conidia had decreased to 320/m.^{3}. The three following rainless days showed concentrations varying between 226 and 640/m.^{3}.



Text-fig. 5. Plantation B. Daily mean concentration of *D. torulosa* conidia related to rainfall and irrigation during period 1 September–5 October 1960.

A detailed 2-hourly analysis of changes in spore concentration during the first irrigation period is given in Text-fig. 6. Periodicity similar to that in plantation A was undergone each day, but numbers of spores trapped greatly exceeded those in the former plantation. The maximum concentration recorded was 72,335/m.^{3} at 08.00 hr. on 8 September. An area of the spore trace corresponding to this time is shown in Pl. 28, fig. 2.

There was a further large increase in concentration on 15 September following 1·22 in. rain on the previous afternoon. At 08.00 hr. a concentration of 57,270/m.^{3} was recorded. On the same day, 0·28 in. rain fell in the afternoon and an additional trace during the night. There was



Text-fig. 6. Plantation B. Relation of rainfall and concentration of *D. torulosa* conidia every 2 hr. during the first under-tree irrigation period in Text-fig. 4.

a heavy shower near 08.00 hr. on 16 September when conidium concentration of 297/m.^{3} was recorded; the daily mean concentration for this date was only 78/m.^{3}. Such low estimates were surprising in view of the high counts normally associated with wet weather. A satisfactory explanation for this unexpected decrease in concentration cannot be presented at this stage, but there are three possibilities. First, rainfall occurring at, or shortly after, the period of most rapid liberation may have removed many spores from the air. Secondly, liberation may have been only slight, due to unfavourable weather conditions; on the date in question, humidity never fell below 80 % and the maximum temperature was 79° F. A third, but seemingly less likely, explanation is that the source of conidia was temporarily exhausted.

Appreciable rainfall on 16, 18, 20 and 22 September was followed by a marked increase in concentration on each following day. On 21 September the estimates at 08.00 and 10.00 hr. were 891 and 6600/m.^{3}, respectively. This was the only recorded occasion on which the peak concentration did not occur near 08.00 hr. Since there had been 0·60 in. rain between 02.00 and 06.00 hr., the plantation humidity was still high (91 %) at 08.00 hr.; by 10.00 hr. it had decreased to 82 %. Thus the period of most rapid liberation once again coincided with that of decreasing vapour pressure, which on this date was 1½-2 hr. later than usual.

The second under-tree irrigation period commenced at 11.00 hr. on 24 September and ended at 17.00 hr. on 26 September. During this period there was an associated increase in daily mean spore concentration, but it was less than that during the previous irrigation period. The highest 2-hourly conidium concentration was 18,117/m.^{3} at 08.00 hr. on 26 September. The period 28 September–20 October was particularly hot and dry, with maximum daytime temperatures exceeding 95° F.; there was a considerable decrease in spore concentration, daily mean values never exceeding 90/m.^{3}. Rainfall on the evening of 2 October resulted in an increased spore concentration on the next day. A further increase occurred during the third under-tree irrigation period, which started at 10.00 hr. on 3 October.

Disease outbreak. As in plantation A, the incidence of spotting was kept under close observation on bunches of fruit that had just shot at the commencement of sampling. There was a very noticeable development of fruit-spot following the first under-tree irrigation period (5–7 September). As was to be expected from earlier studies (Meredith, 1961 *b*), spotting was restricted to the downward sloping, or horizontally orientated, inner sides of the fingers. This distribution is due to impaction occurring chiefly on these uppermost surfaces. Rainfall during the period 7–10 September resulted in an intensification of spotting on all bunches examined. By this time, individual fingers had curved upwards and spots began to appear on the outer sides also. During the above-mentioned periods, intensity of spotting on older fruit increased appreciably, sometimes to an extent which rendered it unfit for sale. There is little doubt that the increased concentration of *D. torulosa* in the air, combined with favourable humidity conditions, were chiefly responsible for this outbreak of disease.

Further slight spotting developed between 11 and 24 September, but a more noticeable increase occurred during the second under-tree irrigation period (24–26 September). Careful observations made on ten bunches during the dry period 28 September–2 October failed to reveal any visible increase in disease severity. The third irrigation period resulted in an appreciable intensification of spotting. Although the fruit under observation was, at the conclusion of sampling, still very immature, it was quite clear that the severity of spotting would result in some of it being rejected, even if there was no subsequent increase in disease.

Surveys of several plantations on the St Catherine plains indicated that severity of fruit-spot in under-tree irrigated plots was consistently higher than in surface-irrigated areas. This had been noticed previously by independent observers (United Fruit (Jamaica) Co., personal communication).

DISCUSSION

The studies reported here indicate that *D. torulosa* is a common component of the air spora of at least two Jamaican banana plantations. The fungus exhibits a very regular diurnal periodicity, starting at 06.00 hr. and reaching a peak near 08.00 hr. Similar periodicity has been observed for *Phytophthora infestans* and *Polythrincium trifolii* in England (Hirst, 1953), for *Nigrospora sphaerica* and other fungi in Nigeria (Cammack, 1955), and for *N.* (?) *oryzae* in Jamaica (Meredith, 1961*c*). Hirst (1953, 1959) has suggested that such regularity may be due to the existence of a definite discharge mechanism, operating under conditions of decreasing vapour pressure. Forcible ejection from sterigmata (Pinckard, 1942) and hygroscopic twisting movements of sporangiophores (De Bary, 1887) have both been suggested in the case of *Peronospora tabacina.* Spore projection accompanying drying has been described for *N. sphaerica* by Webster (1952). In the case of *D. torulosa*, violent discharge of conidia occurs when there is a sudden decrease in humidity, usually between 07.00 and 10.00 hr. each morning. It remains to discover the nature of this discharge mechanism and the ways in which various external factors affect it. Although rain may modify periodicity slightly, it is clearly not a major factor controlling liberation. Similarly, wind appears to be of minor importance, because the period of greatest atmospheric turbulence (12.00–18.00 hr.) corresponds to a period of low concentration in the air of *D. torulosa* conidia.

Observations in the laboratory suggest that production of conidia on dead banana leaves is unlikely to occur when the external humidity is less than about 90%. However, even during predominantly dry weather, the plantation humidity often exceeds 90% for 10–12 hr. during the night. Occurrence of some slight sporulation under such conditions is indicated by the presence of small numbers of conidia of *D. torulosa*; only on a few days were no conidia trapped during the 24 hr. sampling period. On the other hand, wetting of leaves by rain or irrigation water is very favourable for sporulation. This is reflected in the marked increases in spore concentration after rainfall or under-tree irrigation.

It is now clearer why, in certain banana-growing areas of Jamaica, conditions of prolonged rainfall and high R.H. are often followed by a high incidence of Deightoniella fruit-spot. First, the amount of inoculum available for infection increases under such conditions; secondly, a continuous high humidity at the fruit surface will favour spore germination and infection (Meredith, 1961 *b*). Under-tree irrigation also produces conditions favouring sporulation and infection, resulting in a higher incidence of fruit-spot than in surface-irrigated plantations. Although relatively small quantities of rainfall may be followed by a marked increase in the early morning spore content of the air, it is unlikely that severe fruit-spot will develop if there is rapid drying out of the plantation after 08.00 hr.

The average daily mean concentration of *D. torulosa* conidia was higher in plantation B than in plantation A. This may be due partly to the greater amount of banana-leaf 'trash', and hence of conidia of the fungus, in the former plantation. It may be possible to bring about some reduction in fruit-spot by careful, regular detrashing of plantations. Such detrashing would not entirely remove the source of infection, as the top of the leaf sheath also forms a substrate for the fungus; further, some inoculum would still be present on transition leaves, bracts and withering floral parts (Meredith, 1961 *b*). However, detrashing might be sufficient to reduce the incidence of disease to a level at which it ceases to be of great commercial importance. Large-scale experiments are now in progress in Jamaica to test this suggested control measure. It is hoped that spore trapping studies will reveal any decreases in atmospheric spore concentration after detrashing a plantation.

The results of this and of earlier investigations (Meredith, 1960, 1961 *a*, *b*) have emphasized the importance of environmental conditions and of availability of inoculum of *D. torulosa* in relation to development of fruit-spot. However, other factors may sometimes be of greater importance. In the north coastal area of Jamaica, the incidence of banana fruit-spot is generally low throughout the year, contrasting with the situation on the southern plains of St Catherine. In the former area, there is considerably more rainfall, resulting in prolonged periods of high humidity favourable to infection by *D. torulosa*. In addition, recent studies indicate that inoculum of the fungus is as abundant, if not more so, as on the plains (Meredith, unpublished). Possibly growth conditions for the banana plant are better, resulting in increased host resistance. This and other possibilities are being further investigated.

The author is indebted to the Managements of Caymanas Estates Ltd. and of United Fruit (Jamaica) Co., respectively, for providing facilities which made this investigation possible. This paper is published with the approval of the Banana Board, Kingston, Jamaica, W.I.

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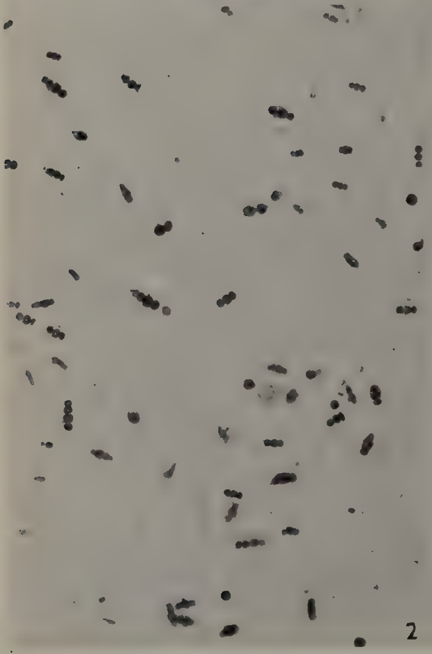

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EXPLANATION OF PLATE 28

Fig. 1. Hirst spore trap in a Jamaican banana plantation.

Fig. 2. Representative field from a portion of the spore trace at 08.00 hr. on 8 September (plantation B), showing many pear-shaped conidia of *Deightoniella torulosa*; a few black, globose or subglobose conidia of *Nigrospora* (?) *oryzae* are present. × 120.

Fig. 3. Conidiophore of *D. torulosa* from a dead banana leaf petiole. The elbowed appearance is due to renewed growth of the conidiophore after spore liberation; this process has occurred several times. × 300.

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ONCOPODIUM PANICI SP.NOV., A HYPHOMYCETE FROM JAMAICA

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(With Plate 29 and 1 Text-figure)

Oncopodium panici on dead culms of *Panicum maximum* is described and briefly compared with *O. antoniae*, the only other described species.

Oncopodium panici sp.nov. (Text-fig. 1 and Pl. 29)

Sporodochia superficilia, gregaria, pulvinata vel hemisphaerica, 92–154 μ diam., cum 30–100 conidiophoris. Conidiophora hyalina, simplicia, continua, 21–33 μ longa, apicem versus ovoidea-inflata, ibique 7·5–12 μ lata; ad basim 3–4·5 μ lata, monospora. Conidia irregulariter pluriseptata, laterite complanata, 17–28 μ longa, 11–16 μ alta, 10–13 μ lata, initia parce clathrata, mox dense murales vel radiatim clathrata, cum pileolo fuligineo et margine hyalino.

Habitat. In culmis emortuis Panici maximi in campo University College of the West Indies, Mona, St Andrew, Jamaica. Typus: Herb. I.M.I. 79057.

Oncopodium Sacc. (1904) was originally characterized by loosely packed, hyaline, continuous and filiform conidiophores. These were conspicuous in that they were swollen into a bladder-like structure beneath the single conidium. Saccardo described the conidia as being subglobose, multiseptate, clathrate, fuliginous and projected on two sides into small pointed cones. The inflated conidiophores reminded him of *Stemphylium inflatum* but the conidia differed in being pointed laterally. He suggested that it be grouped close to the genus *Sporodesmium*. The single species described, *O. antoniae* on *Berberis vulgaris*, had conidiophores formed superficially in punctiform groups or scattered. They were simple or rarely branched at the base, hyaline, 40–50 μ long and inflated ovoid above the centre. They measured 14–15 μ at the widest point and 2 μ at the base. The conidia were superficial, spherical or transversely ellipsoidal, 16–20 × 13–14 μ. At first they were sparsely clathrate and subhyaline but later became densely muriform and fuliginous with small, lateral, subhyaline projections, 3–5 μ long.

Abundant collections of a dictyosporous hyphomycete were made on dead culms of *P. maximum* and two small collections on dead leaves of sugar cane (*Saccharum officinarum*). Dr M. B. Ellis examined the collections on *Panicum* and part of the type collection of *O. antoniae*. He is of the opinion that the two are sufficiently closely related to be included in the same genus. On *Panicum* conidiophores are rarely produced singly but usually grouped into sporodochia. These are formed on the surface of the stem, or more usually on the surrounding leaf sheath. They are flattened, hemispherical, 92–154μ diam., and produce 30–100 conidiophores. The conidiophores are unicellular, hyaline, 21–33μ long and 3–4·5μ wide at the base but swelling like a bladder to 7·5–12μ wide beneath the single terminal conidium (Text-fig. 1 B). The conidia are up to 60-celled and clathrate. They consist of a dome-shaped cap of pigmented cells directly above the conidiophore with a broad rim of colourless cells, 3–6 cells wide. This rim may be absent, or only 1–3 cells wide, at the middle of the

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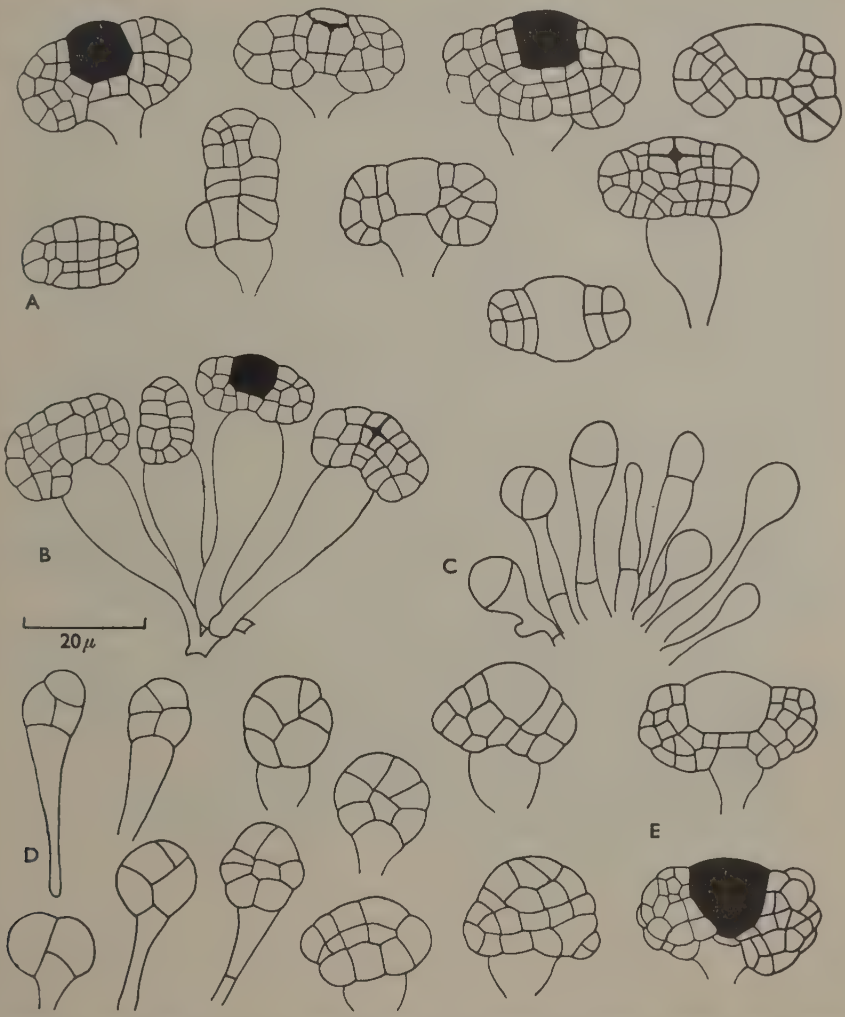
* Now at The Botany School, Cambridge.



Text-fig. 1. *Oncopodium panici.* A, Mature detached conidia; B, attached conidia with inflated conidiophores; C, developing conidiophores and conidial initials; D, developing conidia; E, mature conidia from culture. (A–D from *Panicum maximum.*)

longest side (Text-fig. 1 A, E). The conidia are flattened in the vertical plane and measure 17–28 μ (longest axis), 10–13 μ wide, and 11–16 μ in depth.

The conidia are released from the conidiophores by rupture across the inflated area which leaves a remnant of the conidiophore attached to the conidium. The general shape of the conidiophore would suggest a violent discharge mechanism, as perhaps in *Pilobolus*. Exhaustive attempts to illustrate this however failed.

The conidiophore and conidium initial begins as a large club-shaped cell. The swollen upper region becomes divided by a single transverse septum into an upper conidium primordium and a lower conidiophore (Text-fig. 1 C). The conidium initial soon becomes divided by a further transverse septum (Text-fig. 1 D). The lower of the two cells formed undergoes more rapid cell division in many planes to form the rim of the conidium, while the cell divides less frequently to form the dome-shaped cap. Saccardo (1904) figured the inflated conidiophores and dictyospores of *O. antoniae*, not unlike those described here, except that they appeared more pronouncedly ellipsoidal and there was no cap of darker cells.

Single conidia isolated on oatmeal agar germinated from almost every cell and produced a short, grey-black velvety mycelium with a narrow white margin. Growth was slow, about 3 cm. a month, but by this time sporodochia were forming on the surface as white cushions. Conidia which formed on these were identical with those formed on the host.

I am indebted to Dr M. B. Ellis for his assistance with the identification and for his encouragement. I wish to thank Prof. Capeletti for loaning an authentic collection of *O. antoniae* to the Commonwealth Mycological Institute.

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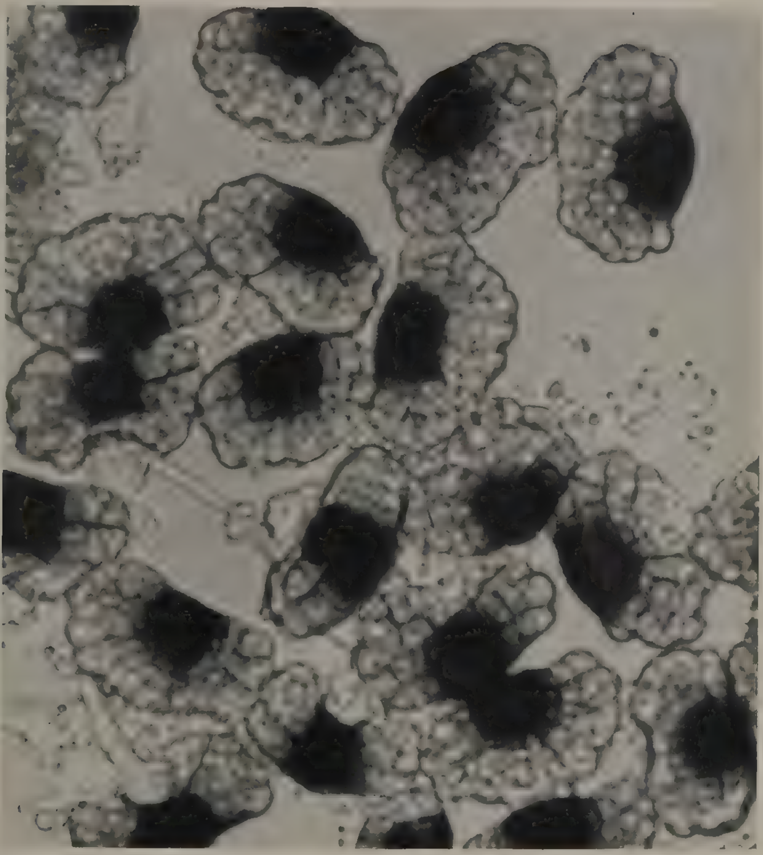
EXPLANATION OF PLATE 29

Fig. 1. Conidia mounted in water. × 1,000.

(*Accepted for publication* 15 *November* 1960)



(*Facing p.* 408)

Trans. Brit. mycol. Soc. **44** (3), 409–416 (1961).

STRAINS OF *PHYTOPHTHORA PALMIVORA* (BUTL.) BUTL. FROM *THEOBROMA CACAO* L.

II. ISOLATES FROM NON-AFRICAN COUNTRIES

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Examination of a further forty isolates of *Phytophthora palmivora*, from fourteen countries outside West Africa, has shown that isolates of the 'cacao' strain in West Africa are similar to those found elsewhere in development and appearance of lesions in cacao pods, sporangial dimensions, and oospore production with sexually complementary strains. West African isolates of the 'rubber' group were unlike isolates from cacao from any other country except in that they formed oospores with 'cacao' group isolates. Most isolates of the cacao black pod pathogen were of the 'cacao' group, although 'rubber' group isolates did occur, and isolates of the 'cacao' group were remarkably uniform, with few exceptions. A survey of 239 isolates of the pathogen from cacao has shown the need for a new method of subdividing the species, and of a fresh complete survey of the fungus.

In an earlier paper (Turner, 1960), an account was given of the occurrence and distribution of strains of *Phytophthora palmivora* attacking *Theobroma cacao* in West Africa. It showed that distinct strains of the pathogen were present which differed in characteristics of development in cacao pods, sporangial dimensions, and oospore production, and that isolates of the two more important strains belonged to the 'cacao' and 'rubber' groups of *P. palmivora*. Another smaller group was atypical of the species. Following this survey, comparison was made with isolates from a number of other cocoa-producing countries to examine the variation and distribution of strains of the fungus, and to compare them with the West African isolates. Results of the comparisons, together with observations on the present method of subdividing the species, are summarized in this paper.

Materials and methods

Isolates of the pathogen were obtained from fourteen different cocoa-producing countries outside West Africa. Pure cultures were received from British Borneo (1), Ceylon (1), Malaya (2), New Guinea (1) and Surinam (2), and pieces of infected material from which the fungus could be isolated were obtained from different localities in Bahia (4), British Guiana (1), Dominica (6), Indonesia (4), Jamaica (3), Panama (3), St Lucia (5) and Western Samoa (5). In addition, cultures were received from the Centraalbureau voor Schimmelcultures, Baarn, of isolates of the 'cacao' group from cacao in Ceylon and Trinidad, sent by Gadd in 1926 and by Ashby in 1929, respectively. Comparative examination of the isolates was made by using the same definitive criteria as were used to typify strains of the fungus in West Africa. Pod tests comprised notes on appearance of the lesion and measurement of its rate of extension, and on development of sporangia and aerial mycelium. Sporangial dimensions were measured for 100 sporangia (washed from pods) of most isolates. Paired cultures of isolates from different countries, and from the same country, were incubated in slope tubes on 4 % oatmeal agar at 16° C., and the cultures examined for the presence of oospores after 3 weeks. Behaviour of the isolates in artificial culture was also noted.

Development of the fungus in cacao pods

Standard inoculation into replicates of ten unripe pods of a single variety was made with pieces of diseased tissue from pods previously inoculated with each isolate, and the length plus diameter of resulting lesions measured after 5 days for statistical analysis. Growth rates in the pods of most isolates showed no significant difference from that of the standard isolate of the typical cacao pathogen at Tafo. Slightly higher rates were shown by one isolate from St Lucia and by the 34-year-old culture from Ceylon, and slightly lower rates were recorded for single isolates from Malaya, British Borneo, Jamaica and Ceylon. Growth rates of the Bahia isolates were considerably lower. The 31-year-old Trinidad isolate was probably not representative in virulence of the pathogen in Trinidad; it might be anticipated that a fresh Trinidad isolate would be as virulent as the 'cacao' group pathogen from other Caribbean localities, which was similar to the Tafo isolate. Comparative data on representative isolates from the different countries are shown in Table 1.

Table 1. *Mean dimensions of lesions produced by representative isolates of* Phytophthora palmivora *5 days after inoculation into unripe cacao pods*

		Lesion size (cm.)			
	Isolate		Ghana S.2 (standard)	Difference (%)	Statistical significance
Bahia	243	17·2	26·0	30	***
British Borneo	162	24·2	27·0	10	***
British Guiana	231	23·8	24·9	4	NS
Ceylon	62	27·3	25·5	7	**
Ceylon	257	24·3	26·0	7	*
Dominica	211	20·6†	20·3†	1	NS
Indonesia	236	27·8	26·8	4	NS
Jamaica	216	17·3	26·0	33	***
Jamaica	218	20·7†	20·3†	2	NS
Malaya	226	24·6	28·8	15	***
New Guinea	71	27·4	27·2	1	NS
Panama	187	26·1	25·2	4	NS
St Lucia	206	24·1	23·5	3	NS
Surinam	229	20·4†	19·9†	1	NS
Trinidad (Y)	63	18·4	25·5	28	***
Western Samoa	188	25·8	25·2	2	NS

(Y), not considered representative; †, lesions in ripe pods; NS, no significant difference, $P = 0{\cdot}5$; *, significant at 5 % level; **, significant at 1 % level; ***, significant at 0·1 % level.

Lesions produced by all isolates alike in cacao pods had regular margins, scattered discrete sporangial masses, and, with the exception of one

Ceylon isolate, an appreciable amount of aerial mycelium; these were characters identical with those associated with isolates of the 'cacao' group from West Africa. Very little aerial mycelium was formed by the Ceylon isolate.

SPORANGIAL DIMENSIONS

All isolates produced abundant sporangia which were of the somewhat elongated form typical of the 'cacao' group, except for the pathogens from British Borneo and British Guiana. Apart from these two isolates, which had similar sporangial length:width ratios, mean sporangial dimensions were very similar for each isolate, and the length:width ratio, a character of some value in classification of West African strains, did not vary at all widely. The ratios of the isolates from Ceylon, Jamaica and Trinidad closely resembled those recorded by Ashby (1929). Comparative sporangial data are summarized in Table 2; it was not possible to examine sporangia of the Bahia and Indonesia isolates. No isolates were found which produced sporangia similar to those of the atypical isolates from Angola and Sierra Leone (Turner, 1960).

Table 2. *Mean dimensions of 100 sporangia from cacao pods inoculated with* Phytophthora palmivora *isolates from non-African countries*

	No. of isolates	Sporangia dimensions (μ)		L:W ratio
		Length	Width	
Ceylon	2	40·0	23·8	1·68
Dominica	6	45·8	25·2	1·81
Jamaica (C)	2	42·0	24·9	1·69
Malaya	2	41·6	24·9	1·67
Panama	3	41·8	24·9	1·68
St Lucia	5	45·7	24·8	1·84
Surinam	2	40·5	24·3	1·67
Trinidad	1	41·6	24·8	1·68
British Borneo	1	45·1	32·7	1·38
New Guinea	1	44·0	25·0	1·76
Western Samoa	5	43·7	25·3	1·73
British Guiana	1	37·5	26·3	1·43
Jamaica (R)	1	43·3	25·0	1·73
West Africa (C)	77	43·4	25·3	1·72
West Africa (R)	99	41·1	31·5	1·30

C, isolates of the 'cacao' group; R, isolates of the 'rubber' group.

OOSPORE PRODUCTION

No oospores were found in any single isolate from any country at the end of the test period. Intercrosses of isolates from within countries almost invariably resulted in no oospore production; when paired cultures of the three isolates from Jamaica were examined, however, no oospores were found in the intercross between two isolates, but they were produced in paired cultures with the third isolate. The latter culture also formed oospores in paired culture with 'cacao' group isolates of West Africa, but not with those of the 'rubber' group. Production of oospores by the other two isolates in paired culture with isolates from outside Jamaica was similar to that of 'cacao' group isolates. The occurrence of the 'rubber' group strain of *P. palmivora* in Jamaica was recorded by Ashby (1929) for an isolate from coconut.

Cultures from all countries were intercrossed, and tests were also made with the West African 'cacao' group isolates from Sierra Leone, Ivory Coast, Ghana, Nigeria, Southern Cameroon, Gabon and Congo; with the 'rubber' group isolates from Nigeria, Southern Cameroon, Fernando Po, Cameroun and Gabon, and with the atypical isolates from Angola and Sierra Leone. Oospore production is summarized in Table 3.

Table 3. *Oospore production in paired cultures of* Phytophthora palmivora *isolates from non-African countries*

	Bahia	British Guiana	Ceylon	Dominica	Indonesia	Jamaica (C)	Malaya	New Guinea	Panama	St Lucia	Surinam	Trinidad	Western Samoa	Jamaica (R)	North Borneo
Bahia	O														
British Guiana	O	O													
Ceylon	O	O	O												
Dominica	O	O	O	O											
Indonesia	O	O	O	O	O										
Jamaica (C)	O	O	O	O	O	O									
Malaya	O	O	O	O	O	O	O								
New Guinea	O	O	O	O	O	O	O	O							
Panama	O	O	O	O	O	O	O	O	O						
St Lucia	O	O	O	O	O	O	O	O	O	O					
Surinam	O	O	O	O	O	O	O	O	O	O	O				
Trinidad	O	O	O	O	O	O	O	O	O	O	O	O			
Western Samoa	O	O	O	O	O	O	O	O	O	O	O	O	O		
Jamaica (R)	X	X	X	X	X	X	X	X	X	X	X	X	X	O	
North Borneo	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O
W. Africa (C)	O	O	O	O	O	O	O	O	O	O	O	O	O	X	X
W. Africa (R)	X	X	X	X	X	X	X	X	X	X	X	X	X	O	O
W. Africa (A)	O	O	O	O	O	O	O	O	O	O	O	O	O	O	O

C, 'cacao' group; R, 'rubber' group; A, atypical; X, oospores formed in paired culture; O, no oospores formed.

Table 4. *Mean diameters (μ) of oospores in paired cultures of* Phytophthora palmivora *isolates after 3 weeks*

	Diameter of oospores (μ)
Ghana/British Borneo	18·4
Nigeria/St Lucia	20·0
Nigeria/Western Samoa	23·4
Nigeria/Panama	19·3
Nigeria/Ceylon	22·5
Nigeria/Ceylon	19·0
Fernando Po/Panama	20·0
Southern Cameroon/Panama	20·1

Oogonia and oospores were similar to those described by other workers as produced by paired cultures of 'cacao' and 'rubber' group isolates, although dimensions were a little smaller on average. Mean diameters of oospores (20 measured) formed in paired cultures of complementary isolates are shown in Table 4.

Almost all non-African isolates were sexually incompatible and belonged to the 'cacao' group of the fungus. Oospores were abundant in paired cultures of the 'cacao' group isolates with one isolate from Jamaica and with the West African 'rubber' group isolates, but were less numerous when grown together with the British Borneo isolate. The 'cacao' isolate from New Guinea also induced only sparse production of oospores with complementary strains. Once again, no oospores were formed in any paired culture containing the West African atypical isolates. Ashby (1929) recorded isolates of the 'cacao' group from cacao in Ceylon, Surinam and Trinidad, and from other hosts in Java.

DISCUSSION

The isolates fell geographically into two zones, those from the Caribbean and South American zone and those from South-East Asia, but no marked difference was found between the two groups of isolates. On agar, all isolates grew readily and appeared similar to the West African 'cacao' group isolates, with the exception of the Western Samoa isolates which were easily recognized by their appearance on culture media. Instead of producing the normal dense covering of aerial mycelium with few sporangia in slope culture, little aerial mycelium was formed, and the surface of the culture was covered by numerous, discrete clumps of sporangia.

One group which might be separated out was that comprising British Borneo, New Guinea and Western Samoa; the isolates of the first two countries induced only sparse oospore formation in paired cultures, and those of the latter country were atypical on culture media. It was not known, however, whether the isolates were typical of the pathogen occurring in British Borneo and New Guinea, as only single isolates could be obtained. The same reservation applied to other countries from which it was not possible to obtain several isolates; hence, there was always a possibility that a single isolate might be atypical for a particular territory. For example, the three isolates from Jamaica were of two strains, the occurrence of which would not have been found with a single isolate.

Although examination of more isolates from several countries would be necessary in order to discover the typical strain occurring there, it was apparent from a broad consideration of the 239 isolates that the cacao pathogens were predominantly of the 'cacao' group of the species. Isolates of this group were found in twenty countries (80 %), of the 'rubber' group in eight (32 %), and atypical isolates were received from two (8 %). These figures represent the results of an examination of isolates from cacao from over one half of the total number of countries where the crop is grown.

The 'cacao' group isolates showed a remarkable degree of uniformity. Small variations in morphological and physiological characters of the fungus might be expected between widely spaced countries; hence, it was surprising that growth rates of isolates from countries as widely separated as Panama, Surinam, St Lucia, Sierra Leone, Congo, Indonesia and New Guinea showed no significant differences from that of the Ghanaian standard isolate, and their sporangial dimensions were also similar. Isolates from both hemispheres almost invariably formed oospores readily in paired culture with sexually complementary isolates. There were a few isolates which did not conform in all characters to typical 'cacao' group isolates. One isolate from Ceylon produced very little aerial mycelium on pod lesions and was the only isolate to differ in this respect, and, similarly, the cultures of the fungus from Western Samoa were unique in having a definitive appearance on culture media. The isolate from New Guinea was unusual in inducing only sparse oospore formation in paired culture with complementary isolates, and oospore production could not be induced in paired cultures of this isolate with the 'rubber' group isolate from Jamaica. One isolate, from British Guiana, had sporangia with dimensions atypical for the 'cacao' group, and rate of lesion development with the Bahia isolates was more characteristic of the 'rubber' group.

Isolates of the 'rubber' group were variable in all characters. One isolate from Jamaica was similar in sporangial dimensions and pod lesion appearance to isolates of the 'cacao' group but differed in other characters, and the isolate from British Borneo did not form oospores readily in paired culture with complementary isolates. The West African 'rubber' group isolates were quite unlike others from any country examined, differing markedly in a number of important characteristics, and they seem to form a distinct, but heterogeneous, group.

Prolonged subjection to low temperatures in sealed cultures was reported by Tucker (1931) to induce oospore formation in single cultures of *P. palmivora*, so all comparisons between isolates of complementary characters were made after a short, defined incubation period. Homothallic isolates of *P. palmivora* from *Areca catechu* were reported by Ramakrishnan & Seethalakshmi (1956), but throughout the present investigation oospores were never seen in single cultures, even in those which had remained untouched for long periods. For *P. palmivora* isolates from cacao, then, oospore production by sexually complementary isolates after a short incubation period is a reliable criterion for strain diagnosis. Single cultures of isolates from rubber also failed to produce oospores.

Gadd (1924) and Ashby (1929) found differences between cultures of the fungus from various hosts, the major difference being based on sexual relationships, as oospores were produced only when sexually complementary isolates were grown together. The species was subdivided on this criterion into 'cacao' and 'rubber' groups, descriptions which have persisted although they do not necessarily indicate specificity for a particular host. This simple division, however, would appear to be inadequate in classification of the strains of *P. palmivora* which occur on cacao alone. Sporangia of the 'cacao' group isolate from British Guiana showed a length: width ratio typical of the 'rubber' group isolates from cacao. The West African 'rubber' group isolates formed a group distinct from all others except for the character of oospore production, and were quite unlike isolates of the same group from Jamaica and British Borneo. The Jamaica and British Borneo isolates would appear to be related from the aspect of complementary characters, and both produced pod lesions typical of the 'cacao' group; the Jamaica isolate also had sporangial dimensions of this group but had the usual lower growth rate in pods of the 'rubber' group. Separation of a group from far-eastern countries was also possible.

Table 5 contains a summary of the diagnostic criteria used in distinguishing strains of *P. palmivora* from cacao from different countries. It was possible to devise a system of classification of isolates of the pathogen examined as follows:

Group A: all isolates of the 'cacao' group which were constant in all characters tested.

- Group A1: isolates which closely resemble group A, but which differ in a small way.
- Group A2: the 'rubber' group isolates which resemble group A in other respects, and which are closely related and sexually complementary to the Group.

Group B: the West African typical 'rubber' group isolates, which are sexually complementary to groups A and A1.

Group C: atypical West African isolates, which may be distinct from *P. palmivora.*

Countries falling within the groups are also shown in Table 5.

Table 5. *Summary of diagnostic criteria used in distinguishing strains of* Phytophthora palmivora *from different countries*

Group		Rate of lesion development	Pod lesion appearance	Sporangial length: width ratio	Oospore formation	Culture appearance
A	Ceylon	C	C	C	C	C
	Congo	C	C	C	C	C
	Dominica	C	C	C	C	C
	Gabon (C)	C	C	C	C	C
	Ghana	C	C	C	C	C
	Indonesia	C	C	—	C	C
	Ivory Coast	C	C	C	C	C
	Jamaica (C)	C	C	C	C	C
	Malaya	C	C	C	C	C
	Nigeria (C)	C	C	C	C	C
	Panama	C	C	C	C	C
	St Lucia	C	C	C	C	C
	Sierra Leone (C)	C	C	C	C	C
	Southern Cameroon (C)	C	C	C	C	C
	Surinam	C	C	C	C	C
	Trinidad	C?	C	C	C	C
A1	Bahia	R	C	—	C	C
	British Guiana	C	C	R	C	C
	New Guinea	C	C	C	C*	C
	Western Samoa	C	C	C	C	A
A2	British Borneo	C	C	R	R*	C
	Jamaica (R)	R	C	C	R	C
B	Cameroun	W	W	W	W	W
	Fernando Po	W	W	W	W	W
	Gabon (R)	W	W	W	W	W
	Nigeria (R)	W	W	W	W	W
	São Thomé	W	W	W	W	W
	Southern Cameroon (R)	W	W	W	W	W
C	Angola	R	A	C	A	A
	Sierra Leone (A)	R	A	C	A	A

A, Atypical of *P. palmivora*; C, characteristic of 'cacao' group; R, characteristic of 'rubber' group; W, characteristic of West African 'rubber' group isolates; groups A, A1, A2, B, C—see text; *, sparse oospore formation.

These groupings of isolates are based only on the criteria tested here which, from the practical viewpoint of testing a range of isolates for pathogenicity to different cacao selections, were largely adequate. However, there is a possibility that use of different criteria, and particularly of physiological ones, might suggest a further subdivision or regrouping of the isolates. Further examination, especially by serological methods, of isolates from as many countries as possible would be both interesting and rewarding.

Orellana (1959) has suggested that strains of *P. palmivora* attacking rubber and cacao should be raised to varietal rank within the species according to their morphology and specific hosts. However, no difficulty was found in infecting cacao pods with isolates of the 'rubber' group from rubber in Ceylon and Malaya, although they produced a different type of lesion; also isolates from cacao in Ghana readily infected rubber fruits. No difference could be found between isolates from rubber and cacao in Ghana (Turner, 1961). In addition, sporangial dimensions of rubber isolates belonging to the 'rubber' group bore more resemblance to those of the 'cacao' group than to those of isolates from any other host. Tucker (1931) reported infection of cacao pods by isolates from a number of hosts other than cacao or rubber. *P. palmivora* has been reported as a parasite of fifty-one genera in twenty-one families of flowering plants (Hickman, 1958); these numbers are probably low, as plant diseases, particularly of plants of no economic importance, have had comparatively little study in the tropical areas where the pathogen flourishes. These considerations alone seem to preclude any subdivision of the species solely on the grounds of host specialization. On the other hand, designation of strains by allotment to either 'cacao' or 'rubber' is inadequate. Occurrence of a number of strains of *P. palmivora* might be expected from the host range and world-wide distribution of the pathogen. If the species were completely surveyed, including isolates from all hosts, it might be possible to devise a system of classification of strains and races based on physiological, morphological and sexual criteria.

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Trans. Brit. mycol. Soc. **44** (3), 417–436 (1961).

OBSERVATIONS ON BRITISH SPECIES OF *PLEOSPORA*. II

By JOHN WEBSTER and MARIA T. LUCAS*
Botany Department, University of Sheffield

(With 10 Text-figures)

Pleospora ambigua, P. media and *P. shepherdiae* are recorded for Britain; conidial states are reported for *P. media* (*Camarosporium* type), *P. shepherdiae* (*Coniothyrium* type) and *P. vitalbae* (*Hendersonia* type); and two new species, *P. lignicola* and *P. submersa*, are described.

7. *Pleospora ambigua* (Berl. & Bres.) Wehmeyer, *Mycologia*, **43**, 42, 1951

Pyrenophora ambigua Berl. & Bres., Micromycetes Tridentini, *Annu. Soc. Alp. trident.* **14**, 44, 1889.

This description is based on Herb. Sheffield no. 2293 on *Reseda luteola*, Stoney Middleton, Derbyshire, June, 1958 (Fig. 1).

Perithecia single, immersed at first, later becoming erumpent, globose or

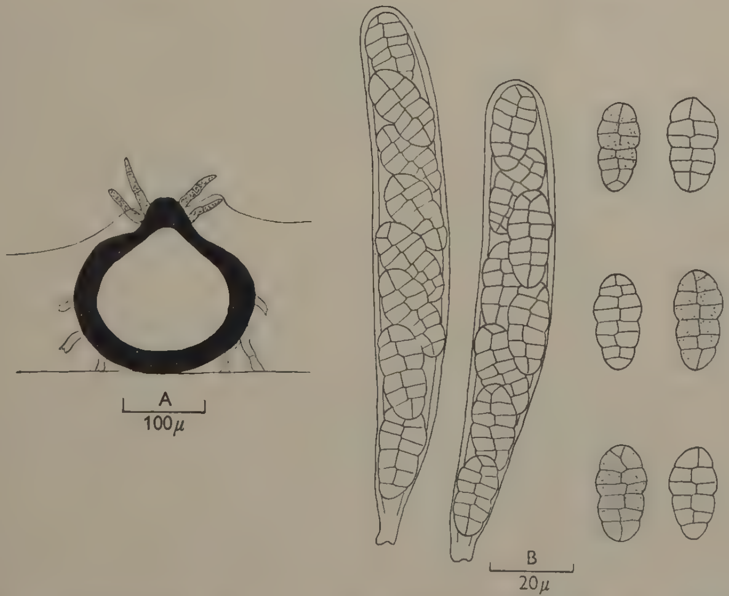

Fig. 1. *Pleospora ambigua* (2293). A, L.S. perithecium on host; B, asci and ascospores.

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depressed-globose, 180–280 μ diam., with flexuous pale brown hyphae at sides and base, and bearing round the papillate ostiole straight divergent pointed dark brown setae, 30–80 μ long, which project through the epidermis; wall up to 40 μ thick, composed of brown polygonal cells. *Asci* club-shaped, bitunicate, short-stalked, 8-spored, 100–130 × 15–20 μ. *Ascospores* uni- or biseriate, oblong-ellipsoidal, symmetrical or with the lower half narrower than the upper, ends obtuse, 5–7 septate (mostly 5), one longitudinal septum in the central cells and often in the end cells, yellow-brown, 20–26 × 10–12 μ.

The specimens match very closely the original description of *Pyrenophora ambigua* Berlese & Bresadola (1889) from *Rumex scutatus*, but we have not seen type material. When Wehmeyer (1951) transferred the species to *Pleospora*, he described two varieties on the basis of the size, the hairy and setose or only setose nature of the perithecia, and spore colour. All our collections match Wehmeyer's description of the typical variety. Prof. L. E. Wehmeyer has kindly confirmed our determinations.

Cultures. Isolates started from single ascospores and single asci and cultured on oat agar slopes grew rapidly forming a yellowish green mycelium at the surface of the medium, with patches of white aerial hyphae. Within 5 weeks, only perithecia were discovered in all isolates. These results agree with those of Simmons (1952, 1954).

The dimensions of other collections on various hosts are listed in Table 1.

Table 1. *Dimensions (in μ) of asci and ascospores of* Pleospora ambigua

Herb. Sheffield	Host	Asci		Ascospores	
		Host	Culture	Host	Culture
2292	*Hieracium* sp.	100–125 × 15–20	100–120 × 16–18	20–24 × 10–12	24–26 × 10–11
2293	*Reseda luteola*	100–130 × 15–20	100–120 × 15–20	20–26 × 10–12	20–22 × 10–12
2378	Umbelliferae	90–120 × 14–16	—	19–22 × 10·5–11	—
2379	*Chrysanthemum leucanthemum*	114–130 × 15–20	—	22–24 × 11–12	—
2380	*Centaurea scabiosa*	90–120 × 15–20	—	21–24 × 11–12	—

8. *Pleospora herbarum* (Fr.) Rabenh. var. *armeriae* Sacc., *Syll. Fung.* **2**, 247, 1883

Sphaeria armeriae Corda, *Icon. Fung.* **4**, 41, 1840.
Pyrenophora armeriae (Corda) Berlese, *Nuovo G. bot. ital.* **20**, 242, 1888.
Pleospora armeriae (Corda) Ces. & de Not., *Schema Sfer. ital.* **1**, 218, 1863.
nec non *P. armeriae* (Rab.) Berlese, *Nuovo G. bot. ital.* **20**, 125, 1888.
P. maritima Rehm 1896 (fide Wehmeyer, 1952*a*).
P. jaapiana Rehm 1907 (fide Wehmeyer, 1952*a*).
Pyrenophora freticola Speg. (fide Wehmeyer, 1952*a*).

The description is based on Herb. Sheffield no. 2051 on *Armeria maritima*, Mannin Bay, Clifden, Co. Galway, Eire, Sept. 1957 (Fig. 2).

Perithecia small, single beneath the epidermis, later bursting through, more or less conical globose, black, 160–200 μ diam.; wall 24–32 μ thick, the outer layer of cells very dark brown, thick-walled, the rest of thin-walled light brown to almost hyaline polygonal cells. *Asci* few (5–10 per perithecium), broadly clavate or ellipsoid, bitunicate, 8-spored, 98–140 × 38–58 μ. *Ascospores* irregularly biseriate, ellipsoid to oblong-ellipsoid, with 7 transverse septa and 1–3 vertical septa in the central cells, slightly constricted at the septa, symmetrical or sometimes with the lower half gently tapered, ends broadly rounded, yellow-brown to dark yellow-brown, 38–45 × 18·5–20 μ.

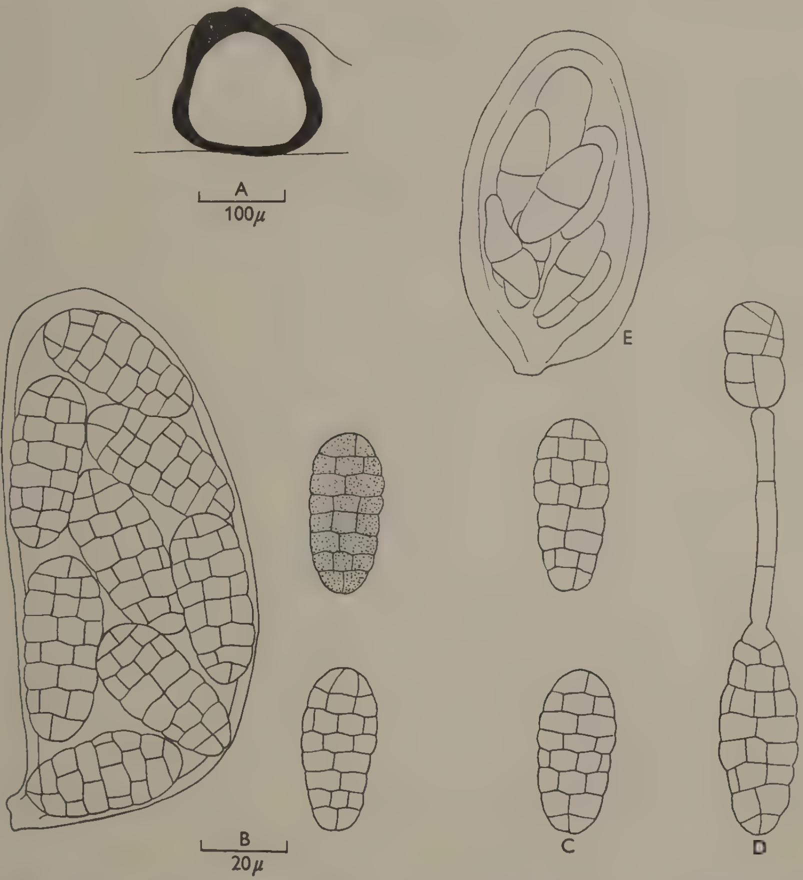

Fig. 2. *Pleospora herbarum* var. *armeriae*. A, L.S. perithecium on host (2051); B, ascus and ascospores (2051); C, ascospores (2294); D, conidium of *Stemphylium* type arising from germinating ascospore; E, immature ascus from the type of *Sphaeria armeriae*. B, C, D, and E to same scale.

Cultures were prepared from single ascospores and single asci from specimen 2294 on maize extract agar. Within a month conidia of the *Stemphylium* type were discovered in association with perithecia on this medium. On oat agar slopes a close felt of dark mycelium was produced at the surface of the medium and an intense black coloration at the upper end of the slopes. Only perithecia were discovered in these cultures when 3 months old.

Similar conidia have been described by many workers from cultures of *P. herbarum* (for references see Wiltshire, 1938, and Wehmeyer, 1955) and the name *Stemphylium botryosum* has been widely accepted for this

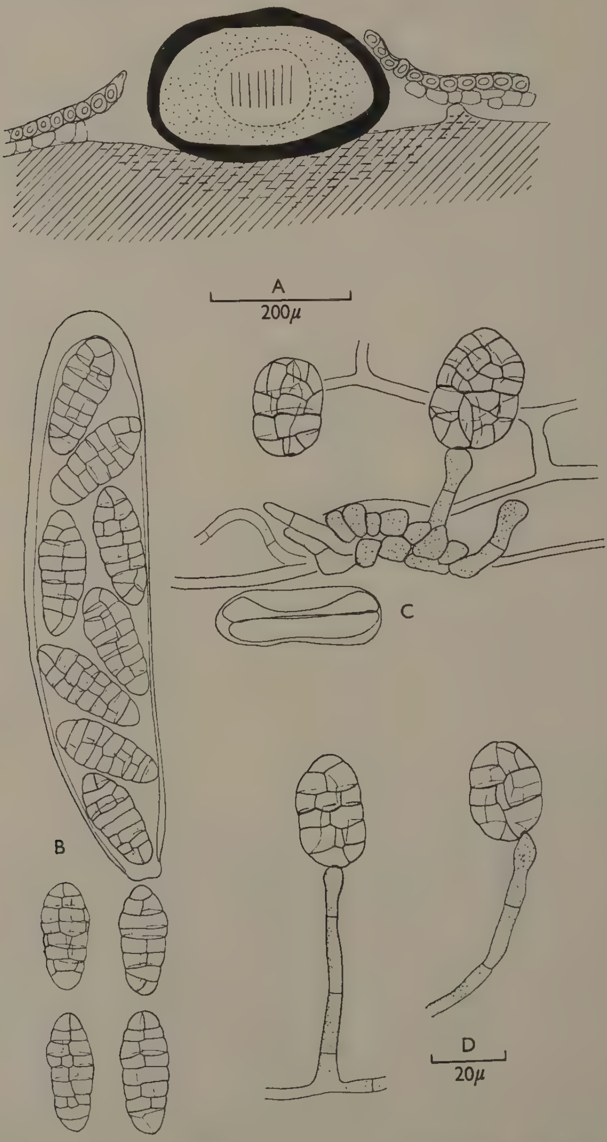

Fig. 3. *Pleospora herbarum* var. *herbarum* (2412). A, L.S. perithecium on *Dactylis*; B, ascus and ascospores; C, conidia on host; D, conidia from culture.

conidial state (Groves & Skolko, 1944). A collection of *P. herbarum* on *Dactylis glomerata* is illustrated (Fig. 3), together with the *Stemphylium* conidia obtained from it in culture (Herb. Sheffield no. 2412).

Sphaeria armeriae Corda was described and figured from flower stalks of *Armeria vulgaris* (= *A. maritima*) in Germany. Cesati & de Notaris (1863) transferred it as *Pleospora armeriae*. Saccardo (1883) treated *P. armeriae* as a form of *P. herbarum*, whilst Winter (1887) and Müller (1951) treat it as a synonym. Berlese (1888, p. 242), however, transferred *S. armeriae* as *Pyrenophora armeriae* (Corda) Berlese and gave (p. 125) *S. armeriae* Rabenhorst (1844) as the basonym for *Pleospora armeriae* (Rabenh.) Ces. & de Not., which he regarded as distinct from the *Pyrenophora*. However, in his description of *S. armeriae* Rabenhorst refers to Corda's earlier description. Clearly there is no such taxon as *S. armeriae* Rabenh., and Corda's name should have priority. Wehmeyer (1952*a*) has discussed this species. His examination of the type specimen showed that it was immature. Through the kindness of Prof. A. Pilát we have seen the type material (Herb. A.C.I. Corda no. 155637) and we also were unable to find fully mature ascospores (Fig. 2). Wehmeyer (1955) has described the development of the ascostroma in three isolates from *Lychnis*, *Arctium* and *Corylus* which he assigns to *Pleospora armeriae*. As far as we know isolates of the fungus from *Armeria* have not previously been compared in culture with *P. herbarum* from other hosts.

Table 2. *Dimensions* (*in* μ) *of asci and ascospores of* Pleospora herbarum *var.* armeriae

Herb. Sheffield no.	Host	Asci	Ascospores
2051*	*Armeria maritima*	98–140 × 38–58	34–45 × 18–20
2294*	*A. maritima*	120–180 × 40–58	36–44 × 19–21
2403	*A. maritima*	110–140 × 38–44	34–36 × 17–18
2404	*A. plantaginea*	90–120 × 30–40	30–34 × 12–16
2405	*A. maritima*	104 × 46	30–36 × 14–18

* Have been grown in culture.

Table 3. *Dimensions* (*in* μ) *of asci and ascospores of* P. herbarum *var.* herbarum

Herb. Sheffield no.	Host	Asci	Ascospores
1964	*Dipsacus sylvestris*	124–140 × 26–30	32–34 × 15–18
2070	*Beta vulgaris*	190–200 × 28–30	29–36 × 14–16
2216	*Epilobium montanum*	150 × 32	30–34 × 14–16
2235	*Barbarea vulgaris*	130–180 × 30–32	34–40 × 16–19
2391	*Armeria plantaginea*	120–140 × 24–28	30–36 × 13–14
2406	*Heracleum sphondylium*	160–180 × 28–30	32–36 × 14–16
2407	*Chenopodium album*	140–160 × 24–30	36–37 × 14–16
2408	*A. plantaginea*	120 × 22	30–38 × 14–16

The evidence from cultures derived from *Armeria* of conidia of the *Stemphylium* type similar to those obtained from cultures of *P. herbarum* on other hosts is, in our opinion, a reason for not separating the two species. However, measurements of asci and ascospores (Tables 2 and 3) of five collections of the fungus on *Armeria* compared with those of eight collections which we assign to *P. herbarum* var. *herbarum* (Table 3) on various hosts including *Armeria* have shown that there is a distinct difference between the two groups of collections in ascus width, the collections assigned to var. *armeriae* having wider asci. Corda's descriptions indicate wide asci in *S. armeriae* and examination of his specimen shows that even the immature asci are broadly sac-shaped, measuring up to 50 μ broad. It is interesting to note that *P. herbarum* var. *herbarum* can grow on *Armeria*, but the distinction in ascus width and that of var. *armeriae* is most striking. The basis for Wehmeyer's (1952 *a*) distinction between *P. armeriae* and *P. herbarum* is the larger spore size. The measurements quoted in his descriptions are:

P. armeriae: asci 110–200 (300 when fully elongated for spore dispersal) × 26–30 μ; ascospores (23) 35–50 × (11) 16–21 μ.

P. herbarum: asci (75) 90–150 (200) × 18–32 μ; ascospores 20–40 × 8–14 (16) μ but always with some spores more than 28 μ long.

9. PLEOSPORA MEDIA NIESSL, *Verh. naturf. Ver. Brünn.* **14**, 188, 1876

The description is based on Herb. Sheffield no. 2049 on *Aster tripolium*, River Nene, near Sutton Bridge, Lincs, July 1957 (Fig. 4).

Perithecia scattered, globose or depressed-globose, 280–320 μ diam., covered with sparse pale brown hyphae, immersed, with the papillate cylindrical-conical ostiole penetrating the epidermis; wall 30–44 μ thick consisting of two different zones of cells, an outer layer of dark brown flattened cells and an inner layer of hyaline polygonal cells. *Asci* numerous cylindrical or cylindrical-clavate, short-stalked, bitunicate, 8-spored 116–130 × 15–19 μ. *Ascospores* irregularly biseriate above, uniseriate below, oblong-ellipsoidal, often asymmetrical, with the lower half narrower and tapered, ends obtuse, predominantly 5-septate, rarely 7-septate, constricted at the three primary septa, with one vertical septum in all central cells and often in the end cells, golden brown, 20–25 × 10–12 μ.

Niessl (1876) based his original description on collections on *Galium mollugo*, *G. verum*, *Echium vulgare* and *Ballota nigra*. We have examined collections from the Botanische Staatssammlung, München, authenticated by Niessl on these hosts which matched our specimen. Wehmeyer (1952) has distinguished a number of varieties, and of these our material matches var. *media* best. Culture studies on var. *acuta* and var. *obtusa* would be of interest. Wehmeyer (1952 *a*) has also listed a number of synonyms of *P. media* var. *media*.

Cultures were prepared from specimen no. 2049. Isolates started from single and multi-ascospores and transferred to oat agar slopes grew rapidly forming a yellowish mycelium with patches of reddish orange aerial hyphae. The substratum was coloured brown. In 3-month-old cultures pycnidia were single, globose 200–320 μ diam., papillate, with thin walls up to 24 μ thick, surrounded by sparse pale brown hyphae. Pycnospores were of the *Camarosporium* type, variable in shape, oblong, ovoid, subglobose, 2- to 3-septate or cruciately septate, yellowish, 8–14 × 5·5–6 μ or up to 10 μ diam.

Specimen no. 2117 on *Suaeda fruticosa* was also grown in culture. On oat agar it produced a deep olive green mycelium with white aerial hyphae. Again pycnidia containing pycnospores of the form-genus *Camarosporium* were discovered within a month. Mature pycnospores were similar in shape to those obtained in culture from specimen 2049 but were larger, 16–20 × 8–10 µ or up to 12 µ diam. and pale brown.

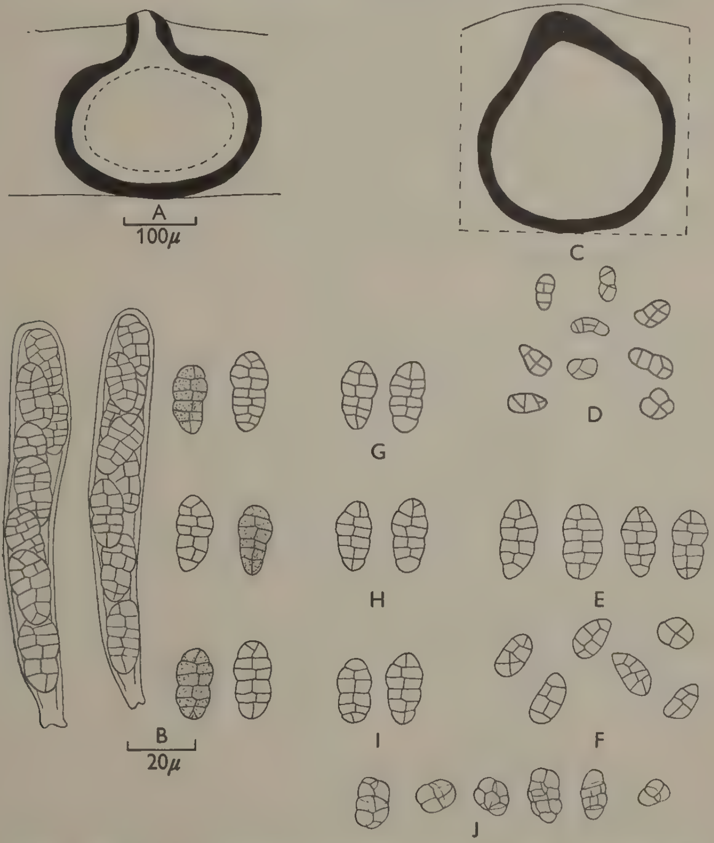

Fig. 4. *Pleospora media.* A, L.S. perithecium on host (2049); B, asci and ascospores (2049); C, section of culture showing pycnidia; D, pycnospores from single ascospore culture (2049); E, ascospores from *Suaeda fruticosa* (2117); F, pycnospores from single ascospore cultures (2117); G, H, and I, ascospores from authentic material of *P. media* on *Ballota nigra. Echium vulgare* and *Galium verum* respectively, from Niessl's Herbarium. J, pycnospores of *Camarosporium punctiforme* from authentic material in Saccardo's Herbarium. B, D, E, F, G, H and I to same scale.

Pycnidia on the host. Similar pycnidia have been found on *Suaeda* (specimen 2117) associated with perithecia. They were globose 200–240 µ diam., papillate, thin-walled. Pycnospores were subglobose, oblong ellipsoidal or ovoid, 2- to 3-septate or cruciately septate, pale brown, 15–20 × 7·5–10 µ or up to 12 µ diam. Cultures started from such pycnospores formed cultures identical with those formed from ascospores. Perithecia have not been found in culture.

Study of published descriptions showed that a large number of species in the genus *Camarosporium* have morphology and dimensions similar to the present isolates, and without examination of a large number of type specimens it is impossible to determine which of these names might apply to the conidial state of *P. media*. It seems likely, however, that a group of species described from Chenopodiaceous hosts might well include *P. media* conidia, e.g. *C. roumeguerii*, *C. halimi*, *C. obionis* and *C. atriplicis*. Hoehnel (1911) regarded these species as synonymous, and proposed that they should be called *C. punctiforme* (Sacc.) Hoehnel. Material of this fungus is preserved in the Saccardo Herbarium at Padua and was borrowed through the kindness of Prof. C. Capelletti. The specimen (1210) is labelled '*Camarosporium* (*Camarosporellem*) *punctiforme* (Sacc.) Sacc. In foliis Atriplicis halimis, Saintes, Gallia (= *Stemphylium* (*Thyrococcum*) *punctiforme* Sacc.).' The leaf fragment bore erumpent black pycnidia, about 150 μ diam. The pycnospores closely resembled those described above (Fig. 4J) and measured 12–21 × 9–12 μ. Such conidia could well belong to *P. media*.

10. PLEOSPORA PALUSTRIS BERLESE, *Nuovo G. bot. ital.*, **20**, 67, 1888

Pleospora aquatica Griffiths, *Bull. Torrey bot. Cl.*, **26**, 443, 1899.

The description is based on Herb. Sheffield no. 2368 on *Scirpus maritimus*, Clenchwarton Marsh, King's Lynn, Norfolk, April 1959 (Fig. 5).

Perithecia immersed, appearing on the surface of the leaf blade as minute black dots, small globose 120–200 μ diam.; wall black, 22–28 μ thick, composed of dark brown polygonal cells. *Asci* broadly club-shaped, bitunicate, 8-spored, 90–108 × 22–28 μ. *Ascospores* irregularly biseriate, oblong-fusoid, symmetrical or more tapered towards the base, 5-septate, constricted at the septa, with one longitudinal septum in all the central cells, but lacking longitudinal septa from the end cells, yellowish, 27–32 × 11–13 μ. When viewed from the side they are flattened, clavate and without a longitudinal septum.

Berlese (1888) cited Cooke, *Fungi Britannici exsicc.* ed. II, no. 689 and Plowright, *Exsicc.* no. 81 as specimens of *P. palustris*. These specimens were originally recorded (Plowright, 1884) as *P. heleocharidis* Karst, and later (Phillips & Plowright, 1887) as *Sphaeria* (*Pleospora*) *heleocharis* Karst. Berlese (1888) claimed that *P. heleocharidis* Karsten (1873) is distinct from the Cooke and Plowright collections and therefore re-disposed them as *P. palustris*. Karsten (1873) cited exsiccatum no. 882, part of which is preserved in Herb. Kew, and examination of this specimen confirms Berlese's opinion (Fig. 5E). The ascospores of Karsten's fungus are dark brown and differ in shape and septation from *P. palustris*. The Cooke and Plowright collections on *Eleocharis palustris* in Herb. Kew have also been examined and, in spite of the discrepancy in size of the ascospores (35–44 × 14–16 μ), their general morphology matches well with our collection. It would be desirable to see further collections to study the range of spore size. Müller (1951) gave spore measurements of 35–42 × 13–16 μ for a collection on *E. palustris*.

Examination of type material of *P. aquatica* Griffiths (1899) from New York Botanic Garden showed that the fungus closely resembles *P. palustris*. Ascospore measurements (29–33 × 11–12·5 μ) match more closely those of our specimen no. 2368. Since *P. palustris* is an earlier name, *P. aquatica* must rank as a synonym.

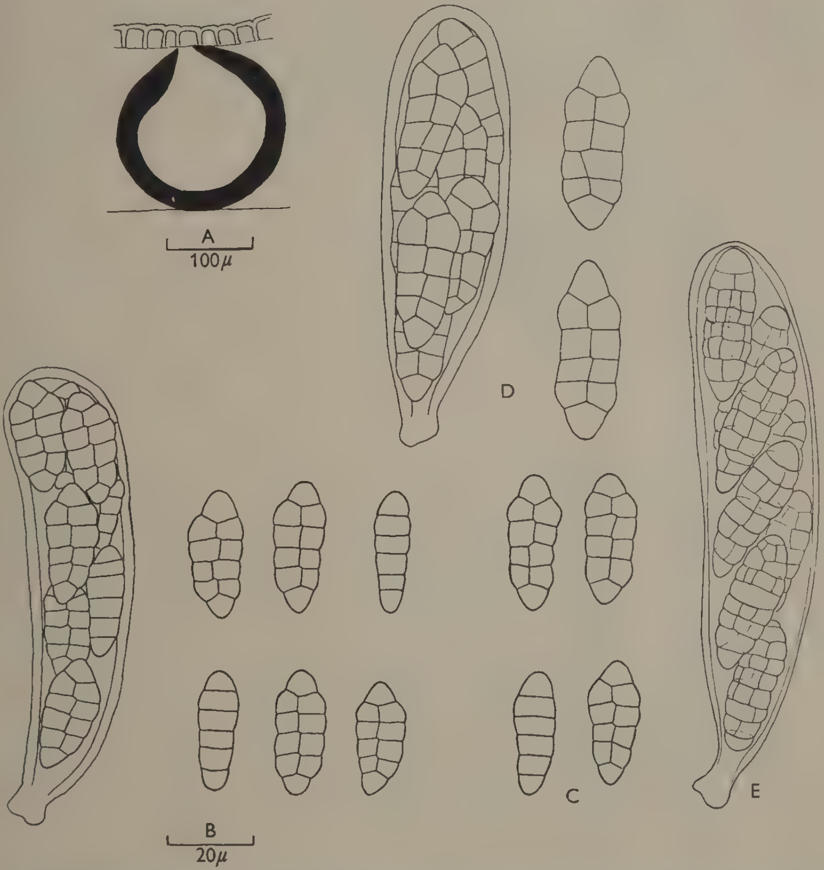

Fig. 5. *Pleospora palustris*. A, L.S. perithecium on host (2368); B, ascus and ascospores (2368); C, ascospores from the type collection of *P. aquatica*, West Amer. Fungi no. 11; D, ascus and spores from Plowright exs. no. 81, the type of *P. palustris*; E, ascus from Karsten's exs. no. 882 of *P. heleocharidis*.

Cultures. Ascospores germinated overnight on maize-extract agar. Isolates started from single and multi-ascospores grew slowly on oat agar slopes, the fungus producing cream to pale grey mycelium. Perithecia were found after 2 months in cultures started from multi-ascospore inocula. Isolates started from single ascospores failed to fruit. Conidia have not been observed.

11. *Pleospora shepherdiae* Peck, *Rep. N.Y. St. Mus.* **40**, 71, 1887

P. carpinicola Ell. & Ev. 1893 (fide Wehmeyer 1949).
Karstenula carpinicola (Ell. & Ev.) Berl. 1900 (fide Wehmeyer 1949).
P. juglandis Ell. & Ev. 1897 (fide Wehmeyer 1949).

The description is based on Herb. Sheffield no. 2093 on dead twigs of *Crataegus monogyna*, Alport, near Youlgreave, Derbyshire, May 1958 (Fig. 6).

Perithecia single or in groups, immersed, prominent, depressed-globose, 320–600 μ diam. with the papillate ostioles penetrating the epidermis; wall uneven in thickness, up to 40 μ thick both at the sides and above, 8–24 μ at the base. *Asci* numerous, cylindrical, short-stalked, bitunicate, 8-spored, 100–124 × 11–13 μ, separated by filiform 'paraphyses'. *Ascospores* uniseriate, oblong-ellipsoidal, symmetrical or with the lower half tapered, ends rounded, 3-septate (rarely 4–5), constricted at the middle, one or two of the central cells with one vertical septum, or rarely in an end cell, light brown, 15–22 × 6·5–9 μ.

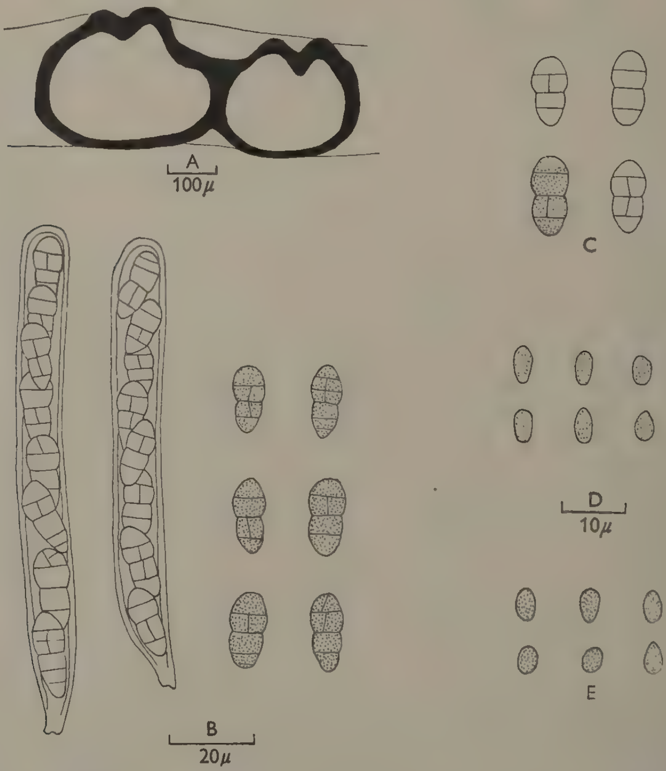

Fig. 6. *Pleospora shepherdiae*. A, L.S. perithecia on host (2093); B, asci and ascospores (2093); C, ascospores from the type collection; D, pycnospores from single ascospore culture (2093); E, pycnospores from host (2093). B, C to same scale; C, D and E to same scale.

The specimen has been matched with the type collection of *P. shepherdiae* from Herb. New York State Museum.

Cultures were prepared on maize-extract agar, and ascospores germinated overnight. Single ascospore isolates were transferred to oat agar slopes, on which a greenish grey mycelium with a great amount of off-white surface mycelium was produced. The medium was coloured pale pink at the margins of the slopes. Pycnidia were discovered in 5 weeks. They are formed singly or in groups, and are globose 120–360 μ diam. with walls 16–32 μ thick and contain pycnospores of the *Coniothyrium* type. Mature pycnospores are ellipsoidal, ovoid or globose, brownish yellow, 4–6 × 2·5–3 μ.

Pycnidia on the host. Similar pycnidia have been found on the host, growing with the perithecia. They are globose, up to 280 μ diam., papillate, brown. The pycnospores are globose, ovoid or ellipsoidal, brownish yellow, 4–6 × 2·5–3·5 μ. Cultures started from pycnospores from the host formed cultures identical with those formed from ascospores.

A large number of species of *Coniothyrium* have been described from woody hosts (Bestagno-Biga, Cifferri & Bestagno, 1958) and we are not prepared to suggest whether any of these might represent conidial *P. shepherdiae.*

12. **Pleospora vagans** Niessl var. **spartinae** var.nov. (Fig. 7)

Differs from the type in having longer asci 150–180 μ × 14–18 μ, and larger, thick-walled spores 24–38 × 10–13 μ. On culms of *Spartina* '*townsendii*', Clenchwarton Marsh, near King's Lynn, Norfolk, 20 August 1956. Type specimen Herb. Sheffield no. 1856.

A typo differt habens ascos longiores 150–180 × 14–18 μ et sporas muris crassis circumdatas, 24–38 μ longas, 10–13 μ latas. In culmis Spartinae townsendii, Clenchwarton Marsh, Norfolk, 20 Augusti 1956. Herb. Sheffield No. 1856 typus est.

The description is based on specimen no. 1856 from Clenchwarton Marsh, near King's Lynn, Norfolk, 20 August 1956, on *Spartina* '*townsendii*'. We have also obtained other collections on the same host from this locality (2020) and from Hole's Bay, Poole Harbour (2061). The perithecial state is most common in late summer on the upper internodes and spike of the *Spartina.*

Perithecia associated with black discoloration of the host tissues, scattered, globose, immersed, penetrating the epidermis by a black, papillate shining ostiole, clothed at the base and sides by pale-brown hyphae; 350–375 μ diam. Wall thick, composed of dark brown polygonal cells. *Asci* cylindrical, pedicellate, rounded above, bitunicate, 8-spored, 150–180 × 14–18 μ, separated by long hyaline filiform paraphyses. *Ascospores* uniseriate, typically 5-septate, constricted at the septa, especially at the middle, with longitudinal septa in the third or fourth cells, or occasionally lacking septa, third cell wider than the rest; spores boat-shaped or slightly inequilateral, thick-walled, yellowish brown, 26–32 × 12–13 μ. The measurements for other collections are: no. 2020, 26–38 × 10–13 μ; no. 2061, 24–31 × 10–13 μ.

Cultures were prepared from single ascospores and asci. Germinated spores were transferred to slopes of oat agar in 6 × 1 in. boiling tubes. On this medium pycnidia (observed after 6 weeks) are globose or depressed-

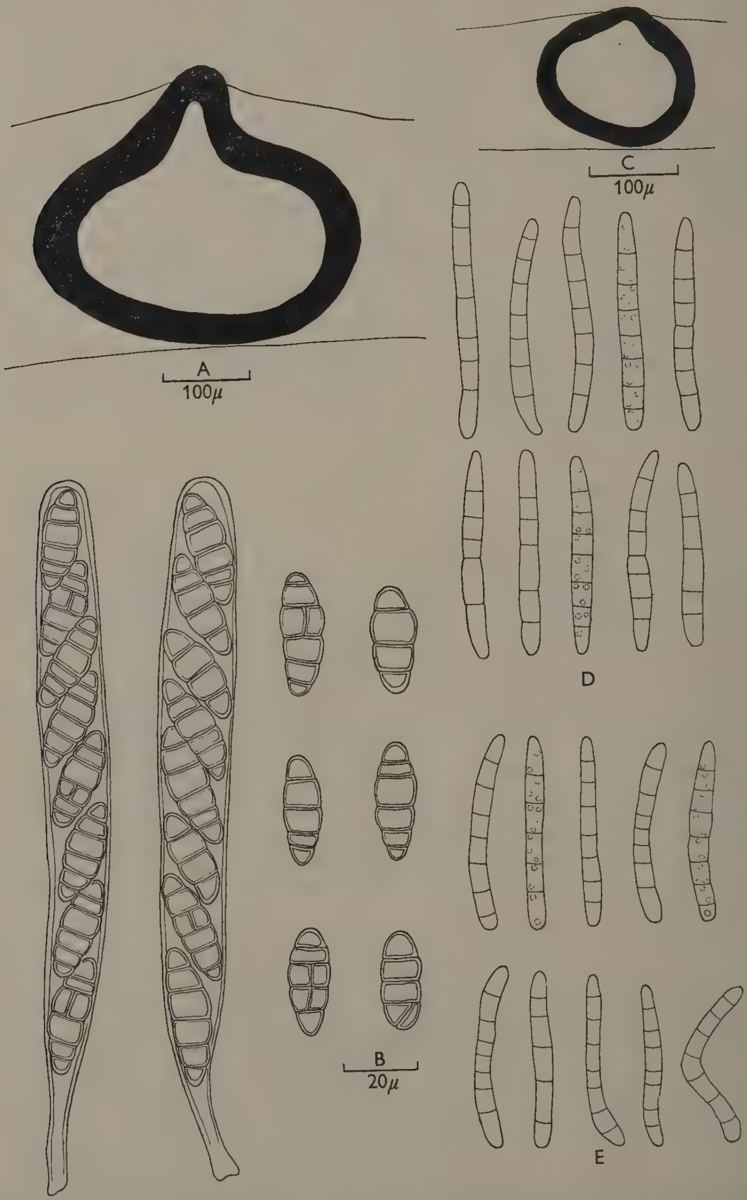

Fig. 7. *Pleospora vagans* var. *spartinae* (1856). A, L.S. host leaf sheath and perithecium; B, asc and paraphyses; C, L.S. host leaf sheath and pycnidium; D, pycnospores from host E, pycnospores from a single ascospore culture on oat agar.

globose, 200–300 μ diam. Pycnospores are cylindrical and tapering at both ends, 5- to 7-septate, faintly constricted at the septa, straight or slightly curved, yellow to pale brown, 36–60 × 3–5 μ. Each cell contains several small guttules grouped near the septa.

Similar pycnidia have been found on the host, and are abundant throughout the year on old leaf-sheaths and blades from upper internodes. Cultures started from such pycnidia matched those started from ascospores.

Pycnidia on the host subepidermal, dark brown to black, globose, surrounded by pale brown hyphae, thick-walled; 160–180 μ diam. The pycnospores resemble those described above and measure 44–72 × 4–6 μ.

When the fungus was first collected the similarity to *P. vagans* was noticed. Comparison with other collections on grasses (Webster, 1955) and on other hosts (Wehmeyer, 1949) showed that the dimensions of the *Spartina* fungus were greater than those of other collections assigned to *P. vagans*. The over-all dimensions tabulated by Wehmeyer and by Webster are compared below with those for all collections of the *Pleospora* on *Spartina*.

Table 4. *Range of dimensions* (μ) *for* Pleospora vagans

	Ascospores	Asci
Wehmeyer 1949	16–26·5 × 5·5–9·5	60–105 × 10–21
Webster 1955	22–30 × 7–11	92–124 × 14–16
Pleospora on *Spartina*	24–38 × 10–13	150–180 × 14–18

The ascospores of the *Spartina* fungus may be longer and are frequently much wider than from other collections, whilst the spore wall is thicker. There are also differences in shape, the typical *P. vagans* spore being distinctly inequilateral, whilst in the *Spartina* fungus this is less obvious. Another feature is that in the latter the formation of longitudinal septa may be tardy, so that spores with only transverse septa are quite frequent. The greater length of the asci of the *Pleospora* on *Spartina* also deserves comment. Another difference is that in cultures of *P. vagans* from a number of grasses perithecia have been found, but not yet in cultures from *Spartina*. In spite of these differences there is close similarity between the conidial states of all collections.

Material of the *Pleospora* on *Spartina* was sent to Prof. Wehmeyer, who kindly examined it and stated that the specimens were 'of the *Pleospora vagans* complex'. We are of the opinion that our material is sufficiently distinct from the majority of collections of *P. vagans* to warrant separation as a new variety of *P. vagans* to which it is very similar. Niessl (1876) has already described three varieties of this fungus.

13. *PLEOSPORA VITALBAE* (DE NOT.) BERLESE, *Nuovo G. bot. ital.* **20**, 70, 1888

Sphaeria vitalbae de Not., *Mem. R. Accad. Torino*, **7**, 32, 1845.
S. vitalbae de Not., *Comment. Soc. critt. ital.* **1** (4), 221, 1863.
Teichospora vitalbae (de Not.) Sacc., *Nuovo G. bot. ital.* **8**, 177, 1876.
Leptosphaeria vitalbae (de Not.) Wint., in Kunze, *Fungi sel. exs.* no. 331, 1880. non *L. vitalbae* Niessl (Tausch Verein 1869) et in Rehm, *Hedwigia*, **27**, 172, 1888.

P. clematidis Fuckel, *Jb. nassau. Ver. Naturk.* **23–24**, 132, 1870.
L. pleosporoides Auerswald, in Rabenhorst, *Hedwigia*, **6**, 88.

The description is based on Herb. Sheffield no. 2348 on dead stems of *Clematis vitalba*, Odell, Beds., February 1959 (Fig. 8).

Perithecia immersed, arranged in rows; globose or depressed globose, black, 280–400 μ diam., and penetrating the epidermis by a well-developed

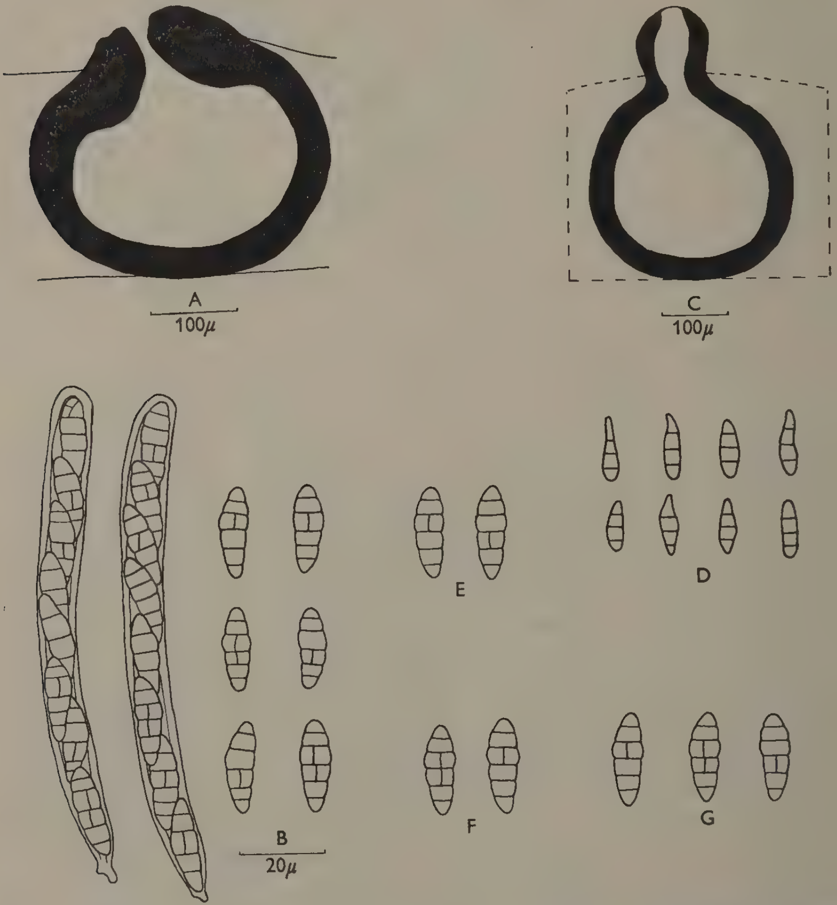

Fig. 8. *Pleospora vitalbae* (2348). A, L.S. perithecium on host; B, asci and ascospores; C, sectio of pycnidium in culture; D, pycnospores from single ascospore culture; E, ascospores from Fuckel, Fungi Rhenani 1799; F, ascospores from Rabenhorst, Fungi Europaei 1253 G, ascospores from Kunze, Fungi Selecti Exsiccati 331.

papillate ostiole which is lined with hyaline periphyses; wall 30–60 μ thick consisting of an outer layer, 7–15 μ thick, of dark compressed cells and a inner layer, 22–40 μ thick, of pale brown polygonal cells. *Asci* numerous cylindrical, bitunicate, 8-spored, 100–130 × 9–10 μ, separated by filamentou pseudoparaphyses. *Ascospores* uniseriate, oblong-fusoid, ends obtuse, 3- t

5-septate (mostly 5), slightly constricted at the septa, a vertical septum in one or both central cells, 3rd cell from the apex enlarged, golden brown, 19–23 × 6–7 μ.

Berlese (1888) cites Fuckel, *Fungi Rhenani* no. 1799, Kunze, *Fungi sel. exs.* no. 331 and Rabenhorst, *Fungi eur.* no. 1253 as exsiccati of his *P. vitalbae*. Portions of these specimens in Herb. Kew have been examined and match closely our material.

Cultures. Ascospores germinated overnight on maize-extract agar. Isolates started from single ascospores grew well on oat agar slopes forming a cream coloured mycelium with white surface hyphae. In 1 month numerous pycnidia were found at the surface of the mycelium. They were single or compound structures, globose 280–560 μ diam., strongly papillate with dark brown walls up to 40 μ thick and surrounded by pale brown hyphae. They contained numerous pycnospores of the *Hendersonia* type. Mature pycnospores are oblong, often tapering towards the apex, or irregularly ellipsoidal, 3-septate, pale brown 12–16 × 3–5 μ and arise from cylindrical hyaline sporophores lining the pycnidium.

H. sarmentorum var. *clematidis* Trav. on stems of *C. vitalba* is a name which should be considered for the possible pycnidial state of *P. vitalbae* but in the absence of pycnidia on the host it is difficult to be certain of this. Saccardo (1913) gives spore measurements as 13–15 × 5–7 μ which match approximately those obtained in culture. We have, unfortunately, been unable to trace type material.

Picbauer (1937) records the occurrence of *P. vitalbae* var. *bulgarica* Bubák & Picbauer on *C. vitalba* in association with *H. bubakii* Picbauer which he described (p. 46) as having 3-septate, brown spores 19–28 μ long, 6–9 μ wide. There is no evidence presented as to whether these fungi are genetically connected with each other. Since *P. vitalbae* var. *bulgarica* has rather larger spores than the type variety of *P. vitalbae* it would be of interest to study the fungus in culture.

14. **Pleospora lignicola** sp.nov. (Fig. 9)

Perithecia superficialia, nigra et nitida, discreta, in superficie ligni, parva (ad 250 μ diametro), globosa, cum ostiolis vix visibilibus. Asci late clavati, octospori, bitunicati, 150–200 × 25–36 μ.

Pseudoparaphyses rarae. Ascosporae biseriatae, fulvae, asymmetricales, superiore dimidio latiore facto quam inferiore, rectae aut inaequilaterales, oblongae aut ad finem tenues, constrictae in medio, cum 7–15 (fere 9) septis transversis et 3–4 septis longitudinalibus in cellis mediis, 34–50 × 16–19 μ.

In ligno quercino mari ejecto, Holme-next-the-Sea, Norfolk. Specimen No. 2413 typus est.

The description is based on specimen no. 2413 collected on oak driftwood at Holme-next-the-Sea, Norfolk, 24 December 1959. The specimen was sent to Prof. Wehmeyer who kindly examined it and suggested that it should be described as a new species.

Perithecia superficial, shining black, discrete, on the surface of the worked wood, small (up to 250 μ diam.), globose, ostioles scarcely visible. *Asci* broadly clavate, 8-spored, bitunicate, 150–200 × 25–36 μ. Pseudoparaphyses scarce. *Ascospores* biseriate, golden brown, asymmetrical, the upper half of the spore distinctly broader than the lower, straight or slightly inequilateral, oblong or tapering towards the end, constricted in the middle, with 7–15 (usually 9) transverse septa, and 3–4 longitudinal septa in the middle cells; 34–50 × 16–19 μ. When mounted in water a distinctive mucilaginous envelope swells up and becomes visible round the spore,

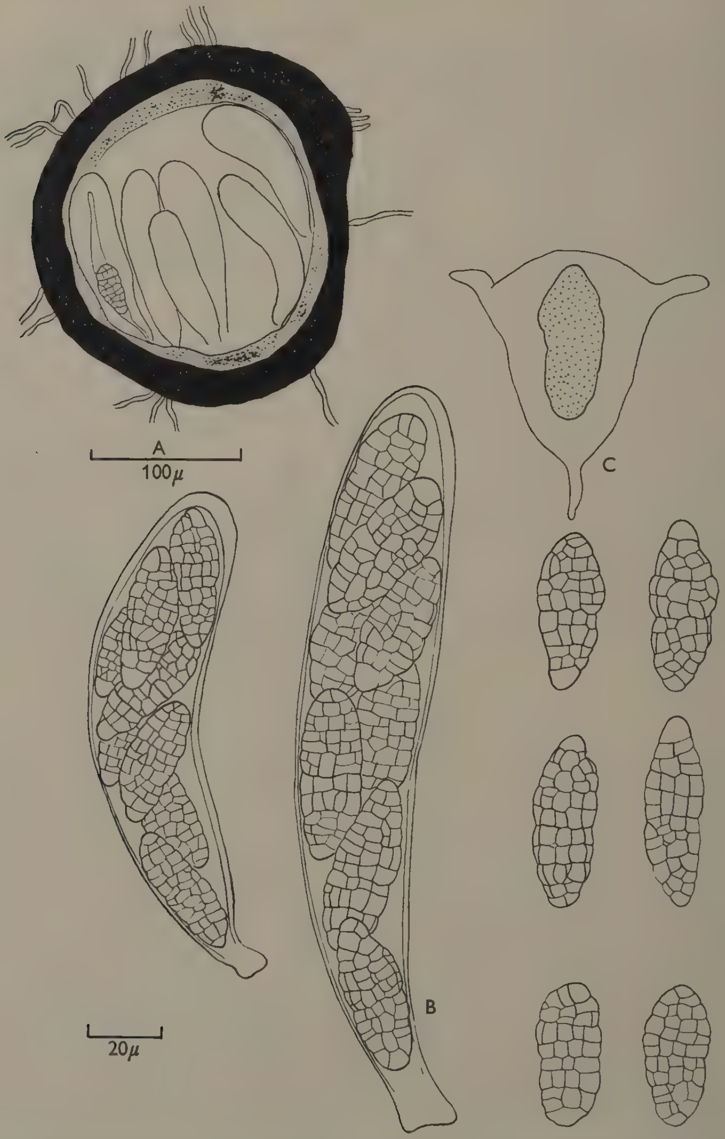

Fig. 9. *Pleospora lignicola* (2413). A, section of perithecium; B, asci and ascospores; C, an ascospore mounted in water to show the mucilaginous sheath with projecting arms.

and projects to form two or three blunt arms (Fig. 9C). Prof. Wehmeyer's description of the spore septation runs '9- 11- 15- septate, mostly 9-septate with three sets of *vulgaris* type septation in each half, but often with incomplete quaternary septa in one or more of the four central cells'.

Cultures single and multi-ascospore cultures were prepared on oat agar slopes, and within 5 months perithecia were found in all isolates. The perithecia resembled those described above. No conidial state was found.

Prof. Wehmeyer (in litt.) writes of this specimen:

> Your fungus on oak wood is also interesting. From the superficial or erumpent superficial position, it would probably be described in *Teichospora*. I am not intimately acquainted with the species of *Teichospora*, but the only species with such small perithecia and many septate spores seems to be *T. sylvana* Sacc. & Speg., on *Vitis*. It does not fit any *Pleospora*. It has the symmetric septation of *P. straminis* or one of my new species *P. muelleri*, but the spores are larger and more septate than *P. straminis* and smaller and more regularly septate than *P. muelleri*. It has small perithecia and biseriate spores which are not characteristic of *Teichospora*; also few pseudo-paraphyses. It could be placed in *Pleospora*.

Comparison with the original descriptions and figures of *T. sylvana* (Sacc. & Speg.) Sacc. (Thümen, 1878; Saccardo, 1878*a*, *b*) show that the fungus is certainly similar to *P. lignicola* but differs in having larger perithecia, longer and wider asci, shorter and wider spores. There is also no mention of the mucilage envelope. Authentic material of *T. sylvana* is preserved in the Saccardo Herbarium (1518, on *Populus tremula*) but examination of this specimen unfortunately proved fruitless.

15. **Pleospora submersa** sp.nov. (Fig. 10)

Perithecia purpurea vel brunnea, globosa, 320–480 μ diametro, ligno operta, quorum ostiola papillata, maculis parvis et nigris similia apparent, per magnem partem ligni putridi purpuri. Asci late clavati, parte superiore rotundi, cum stipitibus tenuibus, bitunicati, octospori, 160–192 × 21–26 μ, paraphysibus filiformibus separati. Ascosporae brunneae, superiore parte asci biseriatae, inferiore parte uniseriatae, oblongae, 7–8 septatae, rectae vel ad finem inferiorem tenues; extrema obtusa, in septis constricta; unum septum verticale in omnibus cellis mediis, nonnumquam in cellis extremis quoque. In ramis submersis Salicis, Esthwaite Water, Lancs. Specimen 2303 typus est.

The description is based on Herb. Sheffield no. 2303 on submerged twigs of *Salix* sp., Esthwaite Water, Lancs, Nov. 1958.

Perithecia purplish to dark brown, globose, 320–480 μ diam., immersed in the wood with the papillate ostioles appearing as small black spots on large purple areas of colonized wood; wall 40–80 μ thick in the upper part, thinner towards the base. *Asci* broadly club-shaped, rounded above, tapering to a stalk below, bitunicate, 8-spored, 160–192 × 21–26 μ, separated by filiform 'paraphyses'. *Ascospores* irregularly biseriate above, uniseriate below or uniseriate only, oblong, 7–8 septate (mostly 7), straight or slightly tapering towards the lower end, ends obtuse, constricted at the septa, one vertical septum in all central cells and occasionally in the end cells, brown to reddish brown, 30–40 × 12–16 μ.

Cultures. Ascospores germinated within 2 days on maize-extract agar. Isolates started from single ascospores and transferred to oat agar slopes grew slowly (about 5 cm. in 10 weeks at room temperature), producing a white mycelium with rose coloured aerial hyphae. The surface was purple and a purple coloration was formed at the margins of the slopes.

Perithecia were discovered in 10-week-old cultures and were globose 320–600 μ diam., papillate, with walls 24–56 μ thick, asci 210–230 × 24–28 μ and ascospores 36–44 × 12–19 μ. No other type of fructification has been seen.

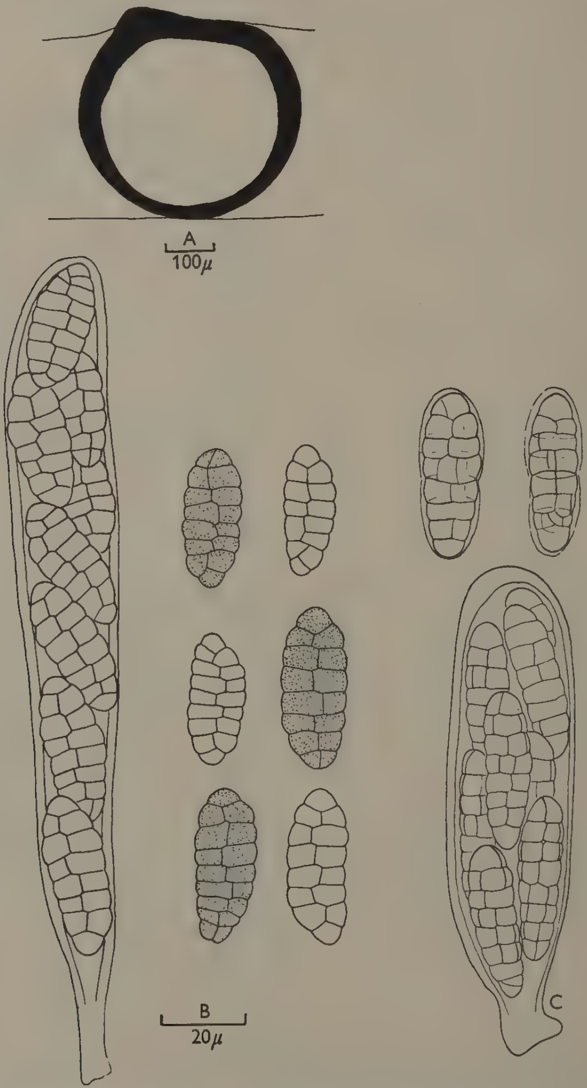

Fig. 10. *Pleospora submersa* (2303). A, L.S. perithecium on host; B, asci and ascospores; C, asci and ascospores from the type collection of *P. henningsiana*.

This species is similar to *P. rubicunda* but is distinguished from it both by its brown to reddish brown oblong ascospores and the absence of a *Phoma* state in culture (Webster, 1957). Specimens and cultures were sent to Prof. Wehmeyer who advised us to describe it as a new species. He wrote: 'These spores are like a new species I have called *Pleospora lutea*, but it has pale yellow spores and small perithecia. Your fungus comes closer to *P. henningsiana*, which is a woody form with larger perithecia, but it has spores which are longer (35–45 × 12–14 μ) and therefore appear narrower. Neither does it have the purple coloration. It is possible that this is a form of *P. henningsiana* which was poorly developed because of being submerged. It is also found on *Salix*.' *P. henningsiana* Ruhland, Jahn & Paul was described by Ruhland (1902) from twigs of *Corylus avellana*, Niederschoenhausen, near Berlin. Type material is preserved in Herb. Sydow, at the Rijksmuseeum, Stockholm. Examination of this showed that *P. henningsiana* is distinct from our fungus, having shorter and wider asci and spores which are proportionately longer in relation to width (Fig. 10C). Wehmeyer (1952*b*) has discussed this species.

Our thanks are due to all those who have allowed us to examine Herbarium material, and to Mr A. O. Hulton who kindly supplied the Latin diagnoses. Miss M. T. Lucas is indebted to the Gulbenkian Foundation for a Research Fellowship, and we are also indebted to the University of Sheffield Research Fund for financial support. Type specimens will be deposited in the Herbarium, Royal Botanic Gardens, Kew.

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POLYPAECILUM GEN.NOV.

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(With 2 Text-figures)

Polypaecilum is characterized by compound annellophores, with several annellated tubes, formed as extensions of a single basal cell. *P. insolitum* sp.nov. is identical with *Scopulariopsis insolita* Coutelen, Biguet, Cochet, Mullet & Doby-Dubois (1955), described without a Latin diagnosis. *P. capsici* comb.nov. is based on *S. capsici* van Beyma (1944).

Polypaecilum gen.nov.

Conidiophora longa aut brevia, irregulariter et laxe confertimve ramosa, diametro inaequali, ramis ultimis duo-plurave annellophora ferentibus, sine septis inter ramum et annellophora; conidia in catenis longis; chlamydospora adsunt aut absunt.
Species typica *P. insolitum* G. Smith.

Colonies slow-growing; conidiophores very irregularly branched with the final branches producing at the tip a number of sterigma-like projections without basal septa, each of which is an annellophore, producing chains of spores. The terminal branches of the sporing structures may be termed compound annellophores. Chlamydospores abundant in *P. insolitum* but not in *P. capsici*.

(Etymology: *πολυποικιλος* = much varied.)

Polypaecilum insolitum sp.nov. (Fig. 1)

Coloniae in omnia media lente crescentes, optime in decocto tuberorum, velutinae sed plicatae, brunneo-griseae, cum margine tenuissima conidiophora ferenta, et centro in maxima parte chlamydospora composito, reverso fusco-atro cum agaro flavo; structurae sporiferae inaequales, compactae aut laxae; conidiophora longa aut brevia, 3–6 μ diam., irregulariter ramosa cum ramis terminalibus duoplurave annellophora ferentibus; conidia globosa subglobosave, subspinulosa, in catenis longis; chlamydospora numerosa, laevia, terminalia aut intercalaria, globosa, ovoidea, pyriformia, aut inaequalia, 6·5–11 μ diam., vel 8–12 × 6–11 μ.
Typus cultura BB. 414 ad L.S.H.T.M. est.

In December 1958 I received from the Commonwealth Mycological Institute a culture (IMI 75202) stated to be a species of *Scopulariopsis*. It had been isolated from the ear of a male patient in United Leeds Hospital, the original culture having been submitted by Dr C. A. La Touche. Examination showed that it is almost certainly identical with *Scopulariopsis insolita* Coutelen, Biguet, Cochet, Mullet & Doby-Dubois (1955), well described and figured by them but without Latin diagnosis. They received their culture from the Faculté de Medicin, Lyon, where it had been isolated from a tumour in the lung of a female patient.

The fungus is not a *Scopulariopsis* as this genus is usually understood. The vegetative hyphae are very variable in diameter, up to about 6 μ, and in old cultures brown. The sporing structures are very irregular, consisting partly of loose branching systems, with branches of fairly regular diameter, 3–4 μ, partly of complex and compact systems of branches of very varying diameter, up to 6 μ, always with the terminal branches bearing at the tip a number of more or less cylindrical outgrowths, which are not individual cells but are continuous with the supporting branch. Each of these outgrowths is an annellophore, producing chains of spores and gradually elongating in the process. The conidia are globose or nearly so, with uneven surface or finely roughened, 3·5–5 μ diam. In addition, numerous terminal or intercalary chlamydospores are formed in the older parts of cultures and liberated by the decay of the supporting hyphae; they are round, pear-shaped, sausage-shaped, or quite irregular, 6·5–11 μ diam., or 8–12 × 6–11 μ.

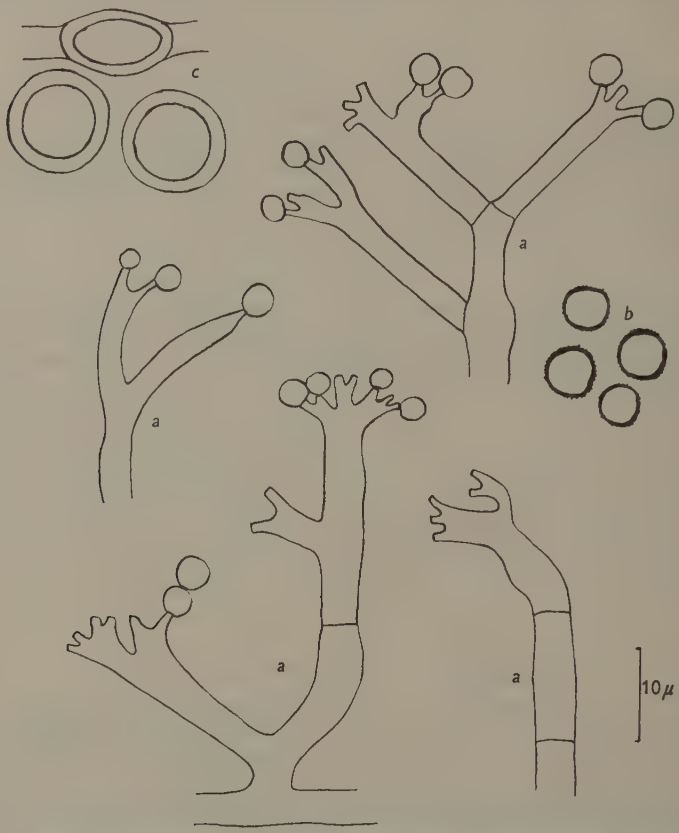

Fig. 1. *Polypaecilum insolitum*. *a*, Conidiophores, showing various types of compound annellophore; *b*, conidia; *c*, chlamydospores.

Growth is slow on all media, but best on potato agar. At 24° C. growth i very slow, restricted, and velvety, but with radial folds and heaped in the centre, somewhat brownish grey, with edge thin, powdery, and of indefinite outline; reverse fuscous-black. At 30° growth is still slow but better than at 24°, brownish grey, velvety, but heaped and convoluted in centre, with edge very thin and partially submerged, bearing patches of a whitish shade consisting mostly of conidiophores, and with centre of colony consisting almost entirely of masses of chlamydospores; reverse fuscous-black but with agar bright yellow.

The type is maintained in culture at L.S.H.T.M. as BB. 414.

Polypaecilum capsici (van Beyma) comb.nov.

Scopulariopsis capsici van Beyma in *Leeuwenhoek ned. Tijdschr.* **10**, 51, 1944.

A subculture from van Beyma's type strain was received from the Centraalbureau voor Schimmelcultures, Baarn, in June 1959. It has the same type of sporing structures as *P. insolitum*, albeit less bizarre. Van Beyma's description and illustration give very little idea of the actual structure of the fungus, so that, until a culture was examined, it was impossible even to guess at its identity.

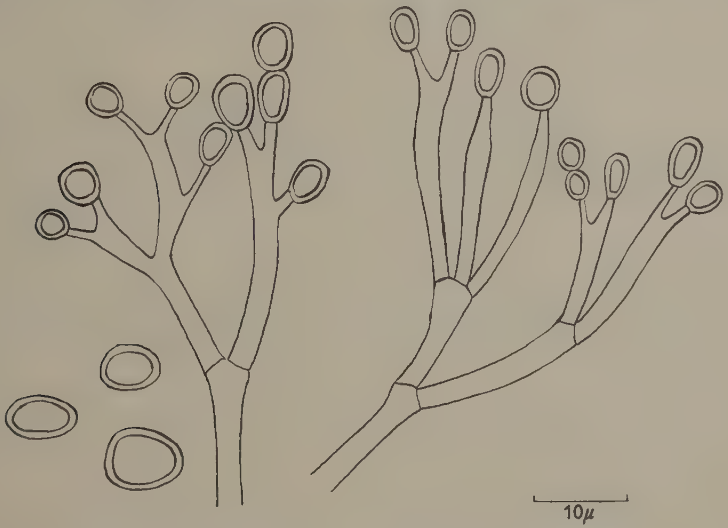

Fig. 2. *Polypaecilum capsici.* Typical conidiophores and conidia.

The fungus grows slowly on all media, best on carrot or potato agar, forming very thick, matted, funiculose colonies, dark brownish grey to nearly black, with white to pale grey edge during the growing period; reverse and agar scarcely coloured. Aerial hyphae almost hyaline, mostly united in funicles; sporing structures consisting of very irregular branching systems, mostly fairly loose and open, with the final branches 2·5–3 μ diam. at base, usually enlarging towards tip, and producing, sometimes terminally, sometimes laterally, two to several narrow prolongations which become annellophores, occasionally with the final branch tapering and terminating in a single annellophore; spores in short chains, thick-walled, irregularly pear-shaped, or almost triangular, usually with a flattened region at point of attachment, 5–9 × 4–7·5 μ, often as broad as long (Fig. 2).

A culture is maintained in the L.S.H.T.M. collection as BB. 423.

Concerning Scopulariopsis americana *Ôta* (1928).

The original description is in Japanese, with a short abstract in French on pp. 16–23. There is no illustration, but figures were published later by Ôta & Kawatsure (1933). These suggest that the affinity of the fungus is with *Polypaecilum* rather than with *Scopulariopsis*. Amongst a batch of cultures received in September 1959 from Instituto Oswaldo Cruz, Rio de Janeiro, was one labelled *S. americana* Ôta, and reported to be ex type. Unfortunately the culture proved to be *Penicillium lilacinum* Thom. Without authentic material to study there can be no certainty as to the placement of this fungus, Hence, for the present, no change of name is proposed. It is, however, of interest that, like *Polypaecilum insolitum*, *S. americana* is pathogenic.

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PITYROSPORUM PACHYDERMATIS WEIDMAN OF CANINE ORIGIN

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(With Plate 30)

Though asporogenous yeasts were numerically greater in otitic than in healthy material, their incidence in healthy and diseased external ears was very similar and it is unlikely that they are of importance as causal agents of ear disease in dogs. They were identified as *Pityrosporum pachydermatis* Weidman by the fact that they multiplied on a broad base, showed no fermentative ability and grew readily on various media without added oleic acid, olive oil or other fatty substances.

During the reproductive cycle, individual oval-shaped cells were seen without polar buds or visible evidence of blastospore formation. All strains hydrolysed urea and grew well on egg media with or without added glycerol or an extract of *Mycobacterium phlei*. They were mostly resistant to antibiotics but were sensitive to high dilutions of certain commercial antiseptics.

A growth-promoting factor for *P. pachydermatis* was produced by some pathogenic and non-pathogenic strains of staphylococci.

Otitis externa, or inflammation of the external auditory meatus, is one of the most common diseases in dogs and is frequently difficult to cure. Many different factors are said to predispose the canine external ear to otitis, including trauma associated with faulty cleaning of the ears, and the presence of wax, foreign bodies and excessive moisture within the meatal canal (Kirk, 1948; Kral & Novak, 1953). The importance of ear mites (*Otodectes cynotis*), which may irritate the lining membrane of the external ear canal, has also been stressed (Jennings, 1953; Kauffmann & Frost, 1949), and it is generally agreed that there is an increased susceptibility to otitis in spaniels and other long-haired breeds with overlapping ears (McGinnis & England, 1949; Joshua, 1958).

Other workers consider that staphylococci, *Pseudomonas aeruginosa*, *Proteus mirabilis* and certain other bacterial species are important causal agents of external otitis (Dam, 1952; Serth, 1954; Gustafson, 1954; Jones, 1955), whereas Schoop (1951) suggested that the disease may be a blastomycosis because many of the affected ears showed a profusion of 'blastomycetes', to the occlusion of bacteria. Gustafson (1955) found an asporogenous yeast, which he named *Pityrosporum canis*, in 70 % of affected canine ears and claimed to have successfully induced the clinical condition in a number of healthy dogs by instilling cultures of the organism into the intact external acoustic meatus. On the other hand, Manktelow (1960) isolated yeast-like organisms, morphologically and culturally identical with *P. canis*, from the ears of 25 % of normal dogs, and Fraser (1957, 1961) reported that the incidence of *Pityrosporum* in healthy and diseased canine external ears appeared to be very similar. In a recent survey of animal mycoses in Britain, Ainsworth & Austwick (1955) found a strain of *P. pachydermatis*, associated with ulceration of the conjunctiva of a dog, which grew well on malt agar without added oleic acid; and they also noted that similar isolates from dogs' ears have been reported by Miss Hoven van Gendern in Sweden.

MATERIALS AND METHODS

Material was collected by means of sterile swabs from the external ear canals of healthy and diseased dogs, inoculated on a variety of media, and incubated in air at 37°. The media, except where stated, were prepared according to standard methods (Mackie & McCartney, 1948).

The antimycotic activity of a number of commercial antiseptics was investigated by the 'ditch-plate' technique, the results being expressed in terms of the highest dilution of antiseptic solution (using tenfold falling dilutions) which completely inhibited growth after 4 days incubation at 37°. Antibiotic sensitivity tests were carried out by adding 'Sentests' to the surface of heavily seeded plates of Sabouraud's agar which were then incubated for 3 days at 37°.

RESULTS

The incidence of Pityrosporum pachydermatis *in canine ears*

Material was examined from the external ears of 277 unselected cases of clinical otitis; from 100 dogs with external ear disease which failed to respond to treatment within 21 days; and from 23 chronically affected dogs which had been suffering from external otitis for at least 3 months, at the time of the first examination, and which were subsequently destroyed. As controls 35 clinically healthy dogs were also examined.

Table 1. *Percentage incidence of* Pityrosporum pachydermatis *and common micro-organisms in canine external otitis*

		Diseased ears		
	Healthy ears (70)	Unselected (400)	Resistant to treatment (151)	Chronic (46)
Pseudomonas	0	13	15	48
Proteus	0	16	17	39
Staphylococcus	54	61	49	57
Pityrosporum	36	44	29	22

The results (Table 1) show that *Pseudomonas* and *Proteus* were present only in otitic material, whereas *Pityrosporum* was almost as frequent in healthy as in diseased external ears. Although *Pityrosporum* was isolated from only 29 % of the external ear canals of dogs which failed to respond promptly to treatment, it was interesting to find that the incidence rose to

36 %, exactly the same as for healthy dogs, following complete clinical recovery.

Of 177 isolates of *Pityrosporum* derived from affected ears, 44 (25 %) were obtained in pure culture, 112 (63 %) were associated with staphylococci, but only 6 (3 %) were present in ears affected by *Pseudomonas* or *Proteus*.

Morphology

Films prepared from otitic material and Gram-stained showed the yeasts as positively staining oval or flask-shaped, double-contoured, single, budding cells approx. 2–3 × 4–7μ (Pl. 30, fig. 1). A similar appearance was obtained in films prepared from solid and fluid cultures except that the transverse septum between the blastospore and the mother-cell was more clearly defined, as were the thick cell capsule and the deeply staining intracytoplasmic granules. Following the release of a fully mature blastospore, many of the yeast bodies had small projections on either side of the mother-cell, immediately below the transverse septum, resembling a protoplasmic collar of residual 'scar' tissue.

The capsulate appearance of the organisms was enhanced by spreading a small quantity of indian ink carefully across the dried surface of a recently prepared Gram-stained culture film. This showed the positively staining cytoplasm to be separated from the background of ink particles by a broad colourless zone which completely encircled both the cell and its blastospore. By Muir's method the cytoplasm was stained purple, red or carmine and was surrounded by a well-defined pale blue capsule which appeared to extend along the broad isthmus between the mother and daughter cells (Pl. 30, fig. 2). Culture films stained by the Ziehl–Neelsen method showed many small, partially acid-fast intracytoplasmic granules, within a larger non-acid-fast mass.

Unsuccessful attempts were made to demonstrate multiple budding and the formation of hyphae by growing the organism on a variety of media under different atmospheric conditions.

Cultural characters

Compared with other members of the genus, *Pityrosporum* of canine origin grew readily in air and in an atmosphere containing 10 % CO_2, also on various fluid and solid media without added oleic acid, olive oil or other fatty substances. The optimum temperature for incubation appeared to be about 37°, but visible growth also occurred at room temperature. Little or no growth occurred anaerobically.

Blood agar. Though most strains failed to grow on 10 % horse blood agar within 4 days, a few primary cultures developed tiny, raised, non-haemolytic surface colonies after 48 hr. incubation at 37°, especially in mixed cultures with staphylococci.

Solidified serum and gelatin media. All isolates produced a slight, raised, pale yellow surface growth on inspissated horse and bovine serum but without liquefaction of the medium even after 6 weeks' incubation. Gelatin slope and stab cultures had small, dry, grey, pitted disks of growth after 4–7 days' incubation at room temperature, and occasional colonies were seen along the length of the stab, to a depth of approx. 3 cm. None liquefied gelatin within 6 weeks, either at 37° or at room temperature.

Sabouraud's agar and malt agar. Most strains produced visible surface growth within 24 hr. at 37°. After 48 hr. incubation a heavy growth was obtained of small, circular, raised, white or buff colonies which reached full size (2–2·5 mm.) within 5 days (Pl. 30, fig. 3). After 6 days at 37° the colonies darkened and became very dry and brittle, while the surface resembled frosted glass and acquired a dimpled appearance not unlike the skin of an orange (Pl. 30, fig. 4).

Corn meal agar. Growth was similar to that on Sabouraud's agar except that the colonies remained chalk white after prolonged incubation.

Nutrient agar. Though an occasional strain grew well on the surface of slopes, the growth of most isolates was scant or absent. A few preferred the addition of 4 % glycerol or 1 % glucose, when a fairly profuse grey growth was obtained.

Glucose and infusion broth media. Though most strains grew poorly in fluid media they invariably produced a characteristic, fine, lace-like, flaky growth on the surface of glucose broth, which settled to the foot of the container with gentle agitation, leaving the supernatant clear. Older cultures frequently produced an adherent, peripheral ring of surface growth, whereas in nutrient broth only a slight granular deposit was observed after 3 days' incubation.

Fluid carbohydrate fermentation media. Growth poor and without visible acidity after 14 days at 37°.

MacConkey's lactose bile-salt medium. All strains grew well, producing pale pink or orange surface colonies of approx. 1·5 mm. diam. after 48 hr. incubation.

Egg media. Good growth was obtained on Dorset's egg and on a modified Finlayson's medium (Taylor, 1950); most isolates grew fairly well on glycerol egg but not on Lowenstein–Jensen medium, with or without glycerol.

Milk media. Only an occasional isolate caused slight reduction of methylene blue milk in 7–10 days at 37°. The majority survived for 14 days but failed to grow in litmus milk for more than 3 days. Most isolates produced visible growth on the surface of 33 % milk agar medium (Christie & Keogh, 1940) within 48 hr., but none hydrolysed milk casein.

Christensen's urea medium (Kauffmann, 1954). All isolates hydrolysed urea within 7 days and most produced visible traces of ammonia after 36 hr. at 37°.

Other reactions. None of the isolates produced indole, H_2S or catalase within 14 days; nitrates were not reduced to nitrites and the Voges–Proskauer and methyl red tests were negative.

Growth-promoting factors of staphylococci

Though *Pityrosporum* from canine sources grew well and could be maintained indefinitely on Sabouraud's maltose or glucose agar and certain other media, a pure growth was seldom obtained in primary culture on 10 % horse blood agar. Moreover, 63 % of the isolates were in mixed culture with staphylococci of which only a few produced coagulase. Growth of *Pityrosporum* was greatly enhanced in the vicinity of colonies of staphylococci which developed on the surface of milk agar plates as a result of aerial contamination during prolonged incubation at room temperature. The presence of a growth-promoting factor was apparently confirmed by the increased growth and larger colonies obtained by streaking *Pityrosporum* across the surface of Sabouraud's maltose agar plates at right angles to a culture of the contaminant staphylococcus (Pl. 30, fig. 5).

Resistance

Though cultures of *Pityrosporum* were maintained for very long periods on a variety of media, most isolates died out after only 4 to 6 weeks' incubation, without subculture. Of 16 isolates grown on an egg medium containing an extract of *Mycobacterium phlei* and maintained at 4° for a year, followed by a further period at room temperature, in the dark, one isolate has survived for at least 2 years 4 months.

Heat resistance. Four-day-old glucose broth cultures were heated for 15 and 30 min. periods at temperatures ranging from 40° to 100° and then plated on Sabouraud's maltose agar. After 4 days at 37° none of the cultures subjected to 50° for 30 min. or 55° for 10 min. survived, but all, with very few exceptions, survived 45° for 30 min. and 50° for 10 min.

Resistance to antibiotics. All isolates were completely resistant, *in vitro*, to penicillin (0·5 i.u.), streptomycin (20 μg.), chloromycetin (40 μg.), bacitracin (100 μg.), aureomycin (10 μg.), terramycin (10 μg.), neomycin (10 μg.), oleandomycin (2 μg.) and erythromycin (1 μg.). Occasionally an isolate was slightly sensitive to 0·1 mg. of sulphonamide and to 2 μg. of novobiocin.

Sensitivity to antiseptics. After the preliminary screening of a number of chemical substances by a modified 'ditch plate' technique, five commercial products were examined more critically for antimycotic activity against *Pityrosporum.* Of the isolates tested, all were completely inhibited by 1 % 'Hibitane', 0·1 % 'Dettol' and 0·01 % 'Tetmosol', 'Roccal' and 'Cetavlon'. A few were also inhibited by 0·001 % 'Cetavlon'.

Reproduction

Though Martin-Scott (1952) considered that the appearance of single, thick-walled spherical cells in *P. ovale* was an optical illusion, due to the bottle-shaped yeasts standing on end, there was little doubt, during this present work, that many of the *Pityrosporum* isolates of canine origin formed individual oval cells as part of their reproductive cycle. This was confirmed by microscopical examination of actively multiplying cells of fresh isolates, maintained in sealed hanging-drop preparations of overnight glucose-broth cultures, at a constant temperature of 37°. Multiplication, by the process of budding on a broad base, continued until the developing blastospore reached the approximate size of the mother cell, when constriction of the cytoplasmic stalk gave rise to two distinct oval bodies surrounded by a common envelope of capsular material. Final separation was achieved by persistent lateral hinge-like movements of the blastospore which clearly demonstrated the flexible nature of the capsule surrounding the paired cells and extending between them in the region of the transverse septum. After a short resting phase both cells began to form blastospores, that of the older cell being produced at the same pole as on the previous occasion.

Non-pathogenicity for laboratory animals

Four-day-old cultures on Sabouraud's maltose agar slopes were applied to the scarified skin surface of mice, rabbits and guinea-pigs and to mice by intravenous inoculation without result.

Discussion

Though Gustafson (1955) found that asporogenous yeasts isolated from cases of canine external otitis closely resembled *Pityrosporum pachydermatis* he suggested, nevertheless, that they be included in *Pityrosporum* Sabouraud as a new species, *P. canis*. It remains to be seen, however, whether the dog strains are sufficiently different from other members of the genus to justify their being elevated to specific rank. In this present work there seemed to be little justification for considering them as a separate species as, apart from the fact that they grew well and were maintained for prolonged periods on a variety of media, their morphological and cultural characteristics were very similar to Weidman's (1925) original description of a yeast which he isolated from the skin of a rhinoceros. For these reasons, and on grounds of priority, we have taken the specific epithet *P. pachydermatis* Weidman to include the strains of canine origin.

The aetiological importance of *Pityrosporum* in canine external otitis was emphasized by Gustafson (1955) who found strains in 70 % of affected ears, compared with only 5 % of healthy ears; and who also reproduced the clinical condition by instilling cultures of *P. canis* into the intact external auditory meatus of healthy dogs. Though Manktelow (1960) isolated asporogenous yeasts from 25 % of the ears of 110 normal dogs, he considered that *Pityrosporum* might play a part in the aetiology of canine external otitis as 43 % of the isolates were recovered from ears containing an excess of discoloured ceruminous secretion. In this present work, asporogenous yeast-like organisms were usually numerically greater in stained films of otitic material than in those prepared from swabs of healthy ears and, though the incidence of *Pityrosporum* in healthy and affected ears was very similar (36 and 44 %, respectively), they were less frequent in chronically affected ears (22 %) and those which failed to respond promptly to treatment (29 %). It should also be emphasized that the relatively high incidence of healthy ears infected by *Pityrosporum* (36 % as compared with 25 % by Manktelow and only 5 % by Gustafson) was not due to the inclusion of subclinical cases of otitis externa in the so-called 'normal' group of dogs, as care was taken to discard all cultures from ears which were subsequently found by histological examination to show hyperkeratinization and hypertrophy of the stratified squamous epithelium of the meatal canal, alteration in the appearance and distribution of the glandular structures in the corium, or other abnormalities which are commonly associated with otitis (Fraser, 1957).

Almost two-thirds of the isolates from affected ears were obtained in mixed culture with staphylococci, many of which were thought to be capable of producing a growth-promoting factor for *Pityrosporum*. It may be that the greatly increased numbers of asporogenous yeasts in otitic material were due partly to the glandular changes in affected ears, providing a more suitable medium for their rapid multiplication, and partly to the beneficial effect on the growth of *Pityrosporum* of staphylococci which were also greatly increased in most acute, non-suppurative cases of canine external otitis. Under these circumstances large numbers of yeasts might possibly irritate the lining epithelium of the external meatal canal, thereby increasing the severity of the clinical condition.

Though isolates of *P. pachydermatis* could not be subdivided according to their cultural and biochemical characters, they were readily distinguished from other members of the genus. Compared with *P. ovale*, which has frequently been isolated from dandruff scales and various skin conditions in man, as well as from healthy tissues, *P. pachydermatis* of canine origin grew well on media without added oleic acid and other fatty substances. They also differed markedly from *P. orbiculare*, a spherical yeast described by Gordon (1951) from the epidermis of human patients, which multiplied on a narrow base and grew well with olive oil or stearic acid but not with oleic acid and mineral oils.

Very occasionally an atypical isolate was encountered which was sensitive to novobiocin, or remained viable on egg media at room temperature for prolonged periods without subculture, or which multiplied by budding with the formation of twin blastospores, one at either pole of the mother cell.

Thanks are due to Dr G. C. Ainsworth and Dr A. Wilson Taylor for helpful criticism and to Mr R. C. W. S. Hood for the photography.

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EXPLANATION OF PLATE 30

Pityrosporum pachydermatis

Fig. 1. Stained film of otitic material (Gram.) × 2200.
Fig. 2. Capsulate appearance in 3-day glucose broth culture (Muir). × 2000.
Fig. 3. Primary culture on malt agar plate (3 in. diam.) incubated at 37° for 3 days.
Fig. 4. Fig. 3 colony, showing pitted surface. × 18.
Fig. 5. Growth-promoting factor. Culture of *P. pachydermatis* inoculated at right angles to a single streak culture of a saprophytic staphylococcus. Maltose agar plate (4 in. diam.), incubated at 37° for 4 days.

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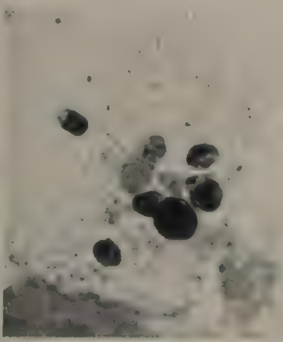

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(*Facing p.* 448)

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CTENOMYCES SERRATUS EIDAM

By DOROTHEA FREY
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(With 1 Text-figure)

An asexual state of *Ctenomyces serratus* has been discovered and is described. The species has been isolated, using a hair-bait technique, from many soils in Australia and New Guinea. The fungus appears to be non-pathogenic to animals.

In recent years we have frequently isolated a keratinolytic moniliaceous fungus from moistened soil specimens using the hair-bait technique (Frey & Durie, 1956). At first the fungus was thought to be the imperfect state of *Thielavia sepedonium* Emmons, owing to the close similarity of the conidia and sporogenous cells, and it was so named by one of us (but see Griffin, 1960*b*, p. 596). However, a comparison of isolates of this fungus with an authentic culture of *T. sepedonium* revealed many differences, principally in gross colony morphology and in the smaller conidial size of the latter. It thus appeared that our isolates might be of an undescribed fungus, and this view was confirmed by Dr C. W. Emmons (private communication).

Recently, however, on one occasion only the conidia were associated with the cleistothecia of *Ctenomyces serratus*. Ascospores from a cleistothecium gave rise, both on Sabouraud's dextrose agar and on soil-hair plates, to colonies bearing first the conidia typical of our hitherto unknown fungus and secondly, after about 1 month, to fertile cleistothecia. There would therefore appear to be no doubt that our conidial isolates represent the previously undescribed asexual state of *C. serratus*, because the conidia attributed to it by Eidam (1880) and Tubaki (1958) were those of *Arthroderma curreyi*, with which *C. serratus* has frequently been confused (Benjamin, 1956; Kuehn, 1958).

Description

Our observations on the sexual state are in complete agreement with those of Benjamin (1956) and Kuehn (1958), both of whom gave descriptions of the cleistothecium and ascospores, well-illustrated by Benjamin (1956) and Swart (1958). We have, however, observed the asci, apparently for the first time.

Asci densely clustered, borne laterally on the very thin ascogenous hyphae, 8-spored, wall evanescent, oval to spherical, *ca.* $4-5\mu \times 5-6\mu$.
Conidia (Fig. 1) obovoid to spherical, hyaline (buff in mass), verrucose to

echinulate (projections *ca.* 1·6μ long), moderately thick-walled, 8·3–16·4μ × 6–12·9μ (average 12μ × 8μ), borne on both aerial and prostrate hyphae, either singly or in botryose masses, sessile (Fig. 1*d*) or on a characteristic clavate to spherical sporogenous cell, each such cell giving rise to 1–3 conidia (Fig. 1*c*, *e*, *f*). Conidia are aleuriospores falling into Section III B. of Tubaki's (1958) modification of Hughes's (1953) classification of conidia and conidiophores. Thus they have a relatively broad insertion on the conidiophore, have continuity between their wall and that of the conidiophore (Fig. 1*d*), and are liberated by the irregular fracture of the hyphal wall. Frequently, a recently liberated conidium thus has an irregular fringe of hyphal wall at its base beyond the delimiting septum Fig. 1*a*). The conidia are therefore similar in developmental type to the micro-conidia of other members of the Gymnoascaceae, e.g. *Gymnoascus gypseus*, and of

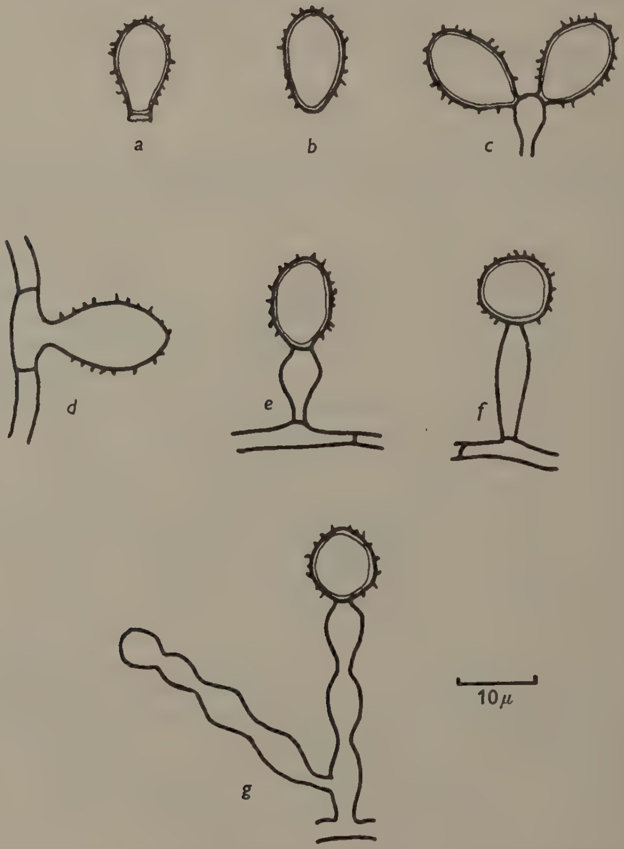

Fig. 1. *Ctenomyces serratus*. *a*, Newly liberated conidium with attached portion of supporting hypha; *b*, old conidium with proximal end rounded; *c*, two conidia borne on a sporogenous cell; *d*, young sessile conidium; *e*, *f*, conidia and sporogenous cells; *g*, anomalous conidiophore with one mature and one developing conidium. (Drawn from mounts in lactophenol cotton-blue.)

Aleurisma carnis. The conidial state would appear to be close to the genus *Sepedonium* Link ex Fr.

On agar young colonies are characterized by an edge of white mycelium but at maturity they are uniformly buff-coloured and powdery due to the abundant conidia; the reverse is colourless to chestnut. The fungus is keratinolytic, hairs being disrupted by splitting, not perforation. Radial growth rate at 25° C. on Sabouraud's dextrose agar in a Petri dish is *ca.* 3 mm./day.

The new isolate has been deposited at the Commonwealth Myclogical Institute, Kew, U.K. (I.M.I. No. 86199), and a subculture will be maintained in the Plant Pathology culture collection of Sydney University (PPUS 255).

Older records of the sexual state of *C. serratus* have been listed by Benjamin (1956) and Kuehn (1958), and there is a recent record from South Africa (Swart, 1958): the most common substratum recorded would appear to be feathers. It is possible that the conidial state was previously found by Cooke (1952) on a wool-cloth bait in contact with soil in the western U.S.A. but he referred the isolate to *Thielavia sepedonium*. Owing to the marked similarity in conidia of the two species and the nature of the substratum, it seems probable to us that the fungus was in fact *C. serratus*. We have isolated the fungus in the conidial state, using hair bait, from soil samples from New Guinea (3 times) and also from New South Wales (3), Queensland (1), South Australia (4), and Victoria (1). It is of interest that all the N.S.W. isolates were obtained from soils associated with birds (two fowl-yards and one nesting-burrow of a White-faced Storm-Petrel, *Pelagodroma marina*). From an ecological survey (by Griffin, 1960*b*), when the fungus was mistakenly described as being asexual *Thielavia sepedonium*, it would appear that *C. serratus* is a less vigorous competitive saprophyte on hair than *Gymnoascus* (*Microsporum*) *gypseus* (Griffin, 1960*a*, *c*), *Keratinomyces ajelloi* or *Trichophyton terrestre*.

No sign of pathogenicity was revealed by skin scarification and inoculation of four guinea-pigs, or subcutaneous inoculation of four guinea-pigs, or intra-peritoneal injection of eight mice.

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NOWAKOWSKIELLA SCULPTURA SP.NOV.

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(With 24 Text-figures)

Nowakowskiella sculptura sp.nov. has been isolated on cellophane from soil collected in Louisiana, U.S.A. It is characterized primarily by predominantly endo-operculate zoosporangia, small zoospores and highly sculptured resting spores which usually develop from pseudoparenchymata and function as prosporangia in germination. *N. macrospora* Karling has been rediscovered in a brackish habitat in Maryland, U.S.A., and grown on cellophane in sea water.

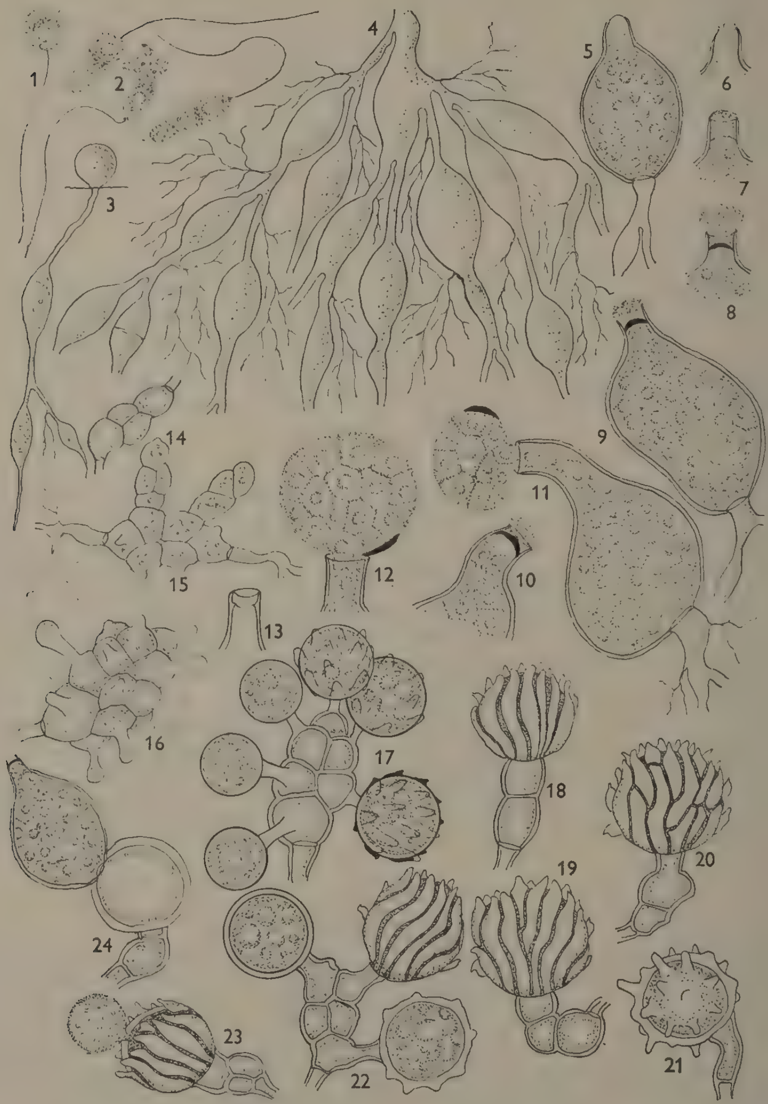
Nowakowskiella sculptura was isolated from soil collected on the campus of Louisiana State University at Baton Rouge, La., in April 1960. A small sample of soil was covered with sterile charcoal-treated water and then baited with floating strips of cellophane which became densely colonized with this chytrid within 6 weeks. Such strips were washed several times in sterile water and after repeated attempts the fungus was subsequently isolated in pure culture. It is characterized chiefly by the development of predominantly endo-operculate zoosporangia on an extensive rhizomycelium, relatively small zoospores, and highly sculptured resting spores, which develop on outgrowths from a pseudoparenchyma which originates by division and extensive proliferation of the spindle-shaped swellings in the rhizomycelium. The structure of the resting spores distinguishes this species quite clearly from the other known members of *Nowakowskiella*.

Nowakowskiella sculptura sp.nov.

Fungus saprophyticus. Rhizomycelio hyalino, profuso, copiose ramoso, partibus tenuibus, 2–8 μ diam., extementis pluribus profuso, non septatis, ovalibus, 8–10 × 14–16 μ, fusiformibus, 7–9 × 12–15 μ, elongatis aut irregularibus. Sporangiis terminalibus aut intercalaribus, hyalinis, subsphericis, 16–36 μ, ovalibus 18–24 × 26–38 μ, pyriformibus, 10–22 × 30–40 μ, oblongatis aut irregularibus; endo-operculo ovalibus aut orbiculatis, 5–8 μ diam. Zoosporis sphaericis, 3–3·8 μ diam., unico globulo refringenti, 0·8–1·2 μ diam.; flagello 16–18 μ longo. Sporis perdurantibus hyalinis, subsphaericis, 13–25 μ, ovalibus, 12–20 × 18–26 μ, sculpturibus, aliquando levibus; germinantibus ut prosporangiis parte interiori emergente ut zoosporangio membrano tenui ad superficiem sporae.

Typus PUL 101 est, legit 21 Apr. 1960, Lafayette, Indiana.

Rhizomycelium profuse, richly branched, tenuous portions 2–8 μ diam., spindle organs unusually profuse in cellophane, usually non-septate, ovoid, 8–10 × 14–16 μ, broadly fusiform 7–9 × 12–15 μ, elongate and sometimes slightly irregular. Sporangia terminal or intercalary, usually non-apophysate, subspherical, 16–36 μ, ovoid, 18–24 × 26–38 μ, broadly or narrowly pyriform, 10–22 × 30–40 μ, oblong or slightly irregular with



Figs. 1–24. *Nowakowskiella sculptura* Karling. 1, Actively swimming zoospore; 2, creeping, amoeboid zoospores, × 1500; 3, germinated zoospore, × 1500; 4, portion of rhizomycelium growing in cellophane, × 300; 5–7, stages in deliquescence of tip of exit tube and formation of endo-operculum, × 800; 8, endo-operculate exit tube with hyaline plug, × 800; 9, 10, relative positions of endo-opercula, × 800; 11, 12, dehiscence of sporangia, × 800; 13, empty exit tube, showing ring-shaped attachment of endo-operculum, × 800; 14, 15, stages in division and proliferation of spindle organs to form pseudoparenchymata, × 750; 16, pseudoparenchyma of 16 cells with spore buds, × 750; 17, pseudoparenchyma bearing 6 young resting spores, × 750; 18–20, commonly sculptured resting spores, × 750; 21, resting spore with few pegs, × 750; 22, pseudoparenchyma bearing a smooth, a sculptured and a verrucose resting spore, × 700; 23, 24, germination stages of resting spores, × 650.

1–2 short or long exit tubes, endo-operculate. Operculum oval or circular in outline and disk-shaped, 5–8 μ diam. Zoospores spherical, 3–3·8 μ, with one hyaline refringent globule, 0·8–1·2 μ diam., flagellum 16–18 μ long. Resting spores frequently occurring in clusters and borne usually on a pseudoparenchyma, hyaline, spherical to subspherical, 13–25 μ, ovoid 12–20 × 18–26 μ, usually with highly sculptured walls, sometimes warty, verrucose, or smooth; functioning as prosporangia in germination.

Saprophytic in decaying debris in soil, Baton Rouge, La., U.S.A.

Type slide PUL 101, coll. 21 Apr. 1960, Lafayette, Indiana, U.S.A.

The vegetative structure, type of development and polycentric organization of the thallus are basically similar to those of other species of this genus and need not be described in detail. The zoospores (Fig. 1) are comparatively small, behave like those of other species (Fig. 2) after being discharged, and germinate (Fig. 3) similarly to produce the rudiments of the rhizomycelium, which develops profusely in cellophane and may form an unusual number of closely adjacent spindle-shaped swellings (Fig. 4). Such swellings are less abundant on the rhizomycelium growing in the water at the edge of the cellophane strips. As the cultures become several months old the walls of these swellings as well as of the tenuous portions become greatly thickened, and sometimes tylose-like plugs of cellulose project into the lumen.

The sporangia are terminal and intercalary and vary markedly in shape and size, as indicated in the diagnosis. Their exit tubes may be short or greatly elongate and tapered at the extremity, and occasionally two or more may develop on one sporangium. As the sporangia and exit tubes attain maturity and the definitive refringent globules begin to form (Fig. 5), the tip of the tube begins to deliquesce (Fig. 6). As this progresses a mucilaginous or viscid-looking mass of substances becomes visible at the tip and extends down into the exit tube for a short distance (Fig. 7). At times this mass appears to be delimited from the sporeplasm by a thin film or membrane (Fig. 7), and it is in this region that the endo-operculum develops (Fig. 8). At times a conspicuous plug of material projects above the operculum (Fig. 8). This plug may gradually diffuse away partly before dehiscence (Figs. 9, 10) or remain intact and be pushed out as the operculum and zoospores are discharged. The endo-operculum may be near the tip of the tube (Fig. 10) or deeper down (Fig. 9). The transformation of the exit tube tip and development of the endo-operculum have been carefully studied in a large number of sporangia in pure culture; in all an endo-operculum was formed in the manner described. However, in a few cases, an exo-operculum developed as I noted (1945) in *N. macrospora* also. As the sporangium dehisces the operculum is pushed out by the emerging zoospores (Figs. 11, 12) and may lie at the top or side of the expanding mass of zoospores. In empty exit tubes the former attachment of the operculum is visible as a ring (Fig. 13).

The resting spores develop from pseudoparenchymatous masses of cells which arise by the extensive division (Fig. 14) and proliferation (Fig. 15) of some of the spindle organs. The cells develop papillae or buds (Fig. 15). These elongate further and swell at their tips to become the rudiments of the resting spores (Fig. 16). Soon, the spores are almost fully developed at the end of short stalks (Fig. 17).

The extensive proliferation of the spindle organs and the frequent production of a spore from each of the cells lead to the occurrence of spores in clusters. Often, several proliferated spindle organs or pseudoparenchymata occur close together, and in such instances as many as fifty or more spores may be found in a cluster. Although this is a frequent occurrence, single and isolated ones (Figs. 18–21, 23, 24) on simple or only slightly proliferated spindle organs may occur also. As the spores mature the walls of the pseudoparenchymatous cells thicken, and the whole structure resembles superficially a sclerotium, though the walls remain hyaline.

The sculpturing of the spore wall begins as the spores approach mature size and first becomes visible as low bumps or peg-like protrusions (Fig. 17). These appear to elongate until the entire spore may be enveloped (Figs. 18–20, 23). However, I am not yet certain just how the development occurs, or what is the exact nature of the sculpturing. On some spores the protrusions seem to curve somewhat and run the full diameter of the spore (Figs. 18, 19, 23), and in other instances they look like narrowly fusiform ridges on the wall. This is particularly visible after such spores have germinated. On other spores (Fig. 20) the pattern of sculpturing may be irregular and broken up; on others the protrusions are reduced to relatively few pegs (Fig. 21) so that the spores are distinctly tuberculate or warty in appearance. Occasionally, only one peg or wart may be visible on the wall, and some spores have a smooth wall (Figs. 22*c*, 24). Accordingly, the degree of sculpturing varies markedly (Fig. 22), a smooth, a warty, and a highly sculptured spore sometimes arising from the same proliferated spindle-organ.

The mature spore contains one to several refractive globules which vary in size and lie in a coarsely granular protoplasm. The spores remain in this condition and appearance until the beginning of germination which may occur within 3–6 months at room temperatures after the spores are mature. In this process the large refractive globules become more highly dispersed, a pore develops in the spore wall, and the protoplasm grows out slowly to form an external sporangium. The resting spore thus functions as a prosporangium in germination as reported for most species of *Nowakowskiella*.

The extensive division and proliferation of the spindle organs to form pseudoparenchymata of different sizes and complexity and the development of the resting spores from their cells in *N. sculptura* are similar to what Butler (1907) and I (1945) described in *N. ramosa* Butl. Also, it is somewhat similar to Domján's (1936) report and figures of spore development in *N. endogena* Constantineanu (1901). In this respect, then, *N. sculptura* appears to be closely related to *N. ramosa*. Its zoospores, however, are considerably smaller than those of *N. ramosa* and also of most of the other nine species reported in the literature of this genus, and more like those of *N. atkinsii* Sparrow. Also, *N. sculptura* resembles *N. macrospora* Karl. and *N. granulata* Karl. in its predominantly endo-operculate sporangia.

Nowakowskiella macrospora Karling

I described this species (1945) from the Amazon Valley in Brazil, I encountered it again in July 1960 while studying the causes of oyster mortalities in the Chesapeake Bay, Maryland. Soil sediment which had collected in a decayed oyster in the Patuxent River at Benedict, Maryland, was flooded with sea water and baited with strips of cellophane. These became densely infected within 4 weeks with *N. macrospora*. It is significant to note that the river water at Benedict is usually highly brackish and that *N. macrospora* colonized the cellophane and grew luxuriantly on it in sea water taken directly from the Chesapeake Bay at Solomons, Md. Apparently, this species occurs in brackish estuarine as well as freshwater habitats. This is not surprising in light of the reports of Gaertner (1955), Harder & Uebelmesser (1955), and Scholz (1958) that numerous freshwater chytrids occur also in brackish soils with high concentrations of salt.

A careful study of *N. macrospora* in Maryland showed its sporangia to be predominantly endo-operculate as I described previously. Sparrow (1960, p. 591) has questioned the presence of opercula in this species on the grounds that he could not find such structures in an otherwise exactly similar fungus which he studied. Apparently, his fungus relates to another polycentric genus, if it is inoperculate.

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PROCEEDINGS, 1961

MEETINGS

11 March 1961. Meeting held at Birkbeck College, London, W.C. 1, at 11.0 a.m. with the President, Miss G. M. Waterhouse, in the Chair.

THE GROWTH AND GENETICS OF THE HIGHER BASIDIOMYCETES

B. E. Plunkett (Birkbeck). Tropisms and formageniture in sporophores of *Polyporus brumalis*.

The influences of the directional stimuli of light and gravity on stipe tropisms and directional aspects of pileus production are considered.

With fixed illumination from one side the stipe is at first positively phototropic, but as the pileus enlarges becomes negatively geotropic. It is shown that the stipe is photo- and geo-tropically competent during both phases. Experiments with changing direction of illumination and artificial 'pilei' of black paper suggest that the change of tropism is due to the shading action of the expanding pileus upon the subpileal photoperceptive and photoreactive region of the stipe.

Stipes and dissepiments grow equally well in either of the two vertical directions, which can be regarded as null-positions for geotropic response. Sporophores inverted as a result of illumination from below during development and others continuously rotated with reference to fixed directions of gravitational and light stimulation have in common that the morphologically upper surface of the pileus always develops towards and approximately at right angles to the direction from which the maximum amount of light is received. Transfer of forming pilei to light of low intensities results in a loss of horizontality and dorsiventral polarity.

G. M. Butler (Birmingham). Growth of vegetative hyphae in *Coprinus disseminatus* and other fungi.

Growth of hyphal branching systems in higher basidiomycetes shows many orderly features, and this paper is concerned to describe some of these features in mycelium of *Coprinus disseminatus* and to consider their implications for possible mechanisms of hyphal growth.

When grown under one set of environmental conditions in Petri dishes, colonies of *C. disseminatus* increased in radius at a constant rate. In the margins of such colonies hyphal growth was essentially monopodial and three groups of hyphae were distinguishable on developmental grounds, namely, main, primary branch and secondary branch hyphae. Hyphae within any one group behaved similarly, but differed markedly, e.g. in tip diameter and extension rate, from hyphae of other groups.

Amongst factors of possible significance in maintaining this branch hierarchy was the occurrence of interactions between separate hyphae via the external medium. Such interactions have been demonstrated in experiments using two-membered cultures separated by permeable cellulose film. Cultures of different ages varied in their ability to induce a reaction in adjacent mycelium of constant age, and the nature of the reaction to the same stimulus depended upon the stage of development of the reacting mycelium.

D. Lewis (University College). The mycelium as a biochemical and genetical unit.

Mycelia consisting of septate hyphae present a number of problems in fungal physiology and genetics. The sphere of action of a nucleus outside its own cell along the hypha, the extent of transport along hyphae of exogenous growth substances and the biochemical differentiation of aerial and submerged hyphae are but three of many unknown quantities. A study of mutants requiring methionine together with their suppressor genes in *Coprinus lagopus* has revealed that in a monokaryotic mycelium the sphere of gene action extends well beyond its own cell, but in a dikaryotic mycelium the same gene has a more limited expression, and probably is confined to its own cell. The supply of exogenous methionine to methionine-requiring mutants is not effectively transported along monokaryotic or dikaryotic hyphae; a fact which may have a direct bearing on the fruiting ability of biochemical mutants. Biochemical differentiation of aerial and submerged hyphae in *Coprinus lagopus* has been found in their ability to grow on acriflavine. Aerial mycelium is able to metabolize much larger quantities than submerged mycelium.

P. R. Day (John Innes Institute). Mating type genes in *Coprinus*.

In *Coprinus lagopus*, mating type is controlled by alleles at two loci *A* and *B*. The total number of alleles is probably of the order of several hundred at each locus. The *A* locus controls clamp formation and the *B* locus controls the migration of nuclei. Both of these processes occur when a dikaryon is formed from two compatible haploid monokaryons.

With the help of linked marker mutants it has been shown that the *A* locus is made up of two subunits which recombine by crossing-over. It has also been possible to isolate mutants in which the function of the *A* locus is impaired. Some progress has been made in the analysis of these mutants. They produce false clamp connexions spontaneously. They may arise by mutation of an unlinked suppressor locus or by changes in or close to the subunits of the *A* locus.

Naturally occurring homothallic forms of this and other species of *Coprinus* have been described. It is suggested that some of these may have arisen by the occurrence of similar mutations.

J. R. Raper (Harvard). An inquiring look at tetrapolar incompatibility.

The genetic basis for tetrapolar incompatibility in the Hymenomycetes has for some years been the subject of intensive and rewarding work. The central problem of how and by what means the incompatibility factors exert their precise and exactly specific roles in the control of mycelial interactions and in the determination of the characteristics of the heterokaryotic products of mycelial interactions, however, remains a tantalizing challenge. Three possible, basic modes of factor action on a constitutive biochemical sequence that tends towards compatibility were discussed in the light of current information concerning genetic and physiological aspects of the incompatibility system: (*a*) *Stimulatory–complementary*, interaction between unlike factors to elaborate an essential component, hence to induce compatibility; (*b*) *inhibitory–oppositional*, interaction between like factors to elaborate an inhibitor, hence to induce incompatibility; (*c*) *inhibitory–competitive*, interaction between unlike factors to prevent the elaboration of an inhibitor, hence to remove incompatibility.

J. H. Burnett (Newcastle). Mating types, individuals and populations in higher fungi.

The present position in respect of numbers and stability of mating-type factors in *Polyporus betulinus*, *Coprinus comatus* and *Nidularia denudata* was outlined and compared with that in other diaphoromictic fungi. At present there seems to be a contrast between bipolar and tetrapolar forms.

The distribution of mating-type factors in local aggregates of *P. betulinus*, *N. denudata*, *Polystictus versicolor* and *Hypholoma fasciculare* will be described. The bearing of these observations on the notion that the mycelium of a higher fungus may be a single ecological unit but a genetical mosaic will be mentioned.

The existence of 'sterility barriers' in *P. betulinus* and *N. denudata* was described, and attention was drawn to comparable cases in other higher fungi and to the nature of mechanisms which isolate populations of closely related fungi.

REVIEWS

Sabouraudia. Vol. 1, Part 1. Edited by J. C. GENTLES. (Edinburgh: E. and S. Livingstone, Ltd., January 1961.) Price: £4 per volume or £1 per part.

After many years of sublimation owing to Man's preoccupation with the natural history and control of diseases of bacterial origin, medical and veterinary mycology have at last asserted themselves and assumed their rightful status. Their belated recognition has been accelerated by the introduction of sulphonamides and antibiotics in the therapy of disease of bacterial origin, for, with their use, many formidable obstacles to disease control have been swept aside, thus clearing the field for the study of problems connected with disease of mycotic origin. Keeping pace with these recent therapeutic developments has been great progress in diagnostic facilities and methods which has made possible a far greater degree of accuracy in the diagnosis of mycotic disease. Indeed, the numbers of reports and dissertations on diseases of fungal origin and on other topics of interest to medical and veterinary mycologists have increased so rapidly in recent years that the few journals in circulation which are devoted solely to the recording of such articles have been quite incapable of absorbing more than a very small proportion of them, the result being a regrettable dispersal of such information in the general medical press or in obscure journals which are accessible to only a small proportion of the readers who might be interested in medical mycology. The advent of *Sabouraudia* is therefore doubly welcome, not only because it is the long awaited official literary organ of the International Society for Human and Animal Mycology, but because it provides a new channel to draw off some of the overflow of literature which is concerned with these topics.

The members of the Society are fortunate in having the services of so able and experienced a mycologist as Dr J. C. Gentles (Great Britain) to carry out the task of editing this Journal for them, and in having for their editorial board such eminent mycologists from other countries as: W. Kaplan (U.S.A.), P. N. Kashkin (U.S.S.R.), C. da Silva Lacaz (Brazil), F. Mariat (France), A. Gonzales-Ochoa (Mexico), H. P. Seeliger (Germany), T. Tsuchija (Japan) and G. A. de Vries (Netherlands).

The first issue of *Sabouraudia* made its appearance in January of this year. Its content will surely leave no doubt in the minds of readers as to the mycological standard expected of contributors.

Most of the articles show results of hard work based on sound mycological principles, thus providing a solid foundation for a journal which, it is to be hoped, will become the foremost exponent of all that is most worthy of publication in the international field of medical and veterinary mycology. Here are professional medical and veterinary mycologists at work, revealing, not only a comprehensive knowledge of the literature of their subject, but also, in every paragraph, reporting their results with an intimate understanding which derives only from a sound mycological education.

The topics which form the subjects of these papers are of the greatest importance to all progressive medical and veterinary mycologists, and the authors are to be congratulated on having provided readers with so much information which is not only new but also fundamental.

Some workers in medical and veterinary mycology may hold the view that the mycological papers in this issue are written in language understood only by mycologists. This in some degree is true, but is inevitable in the interests of accurate recording of mycological facts. It serves no useful purpose to oversimplify accounts of mycological phenomena at the expense of accuracy in order to reach a wider reading public.

It is to be hoped that the standard set by this first issue, no less in the high quality of its illustrations than in the sterling quality of its texts, will be maintained in future issues.

C. J. LA TOUCHE

Traité de Botanique Systématique. By M. Chadefaud and L. Emberger. Tome I. *Les Végétaux non Vasculaires* (*Cryptogamie*). By M. Chadefaud. (Paris: Masson et Cie, 1960.) Pp. xv + 1018, 713 text-figures. Price: 185 NF (paper-back), 200 NF (cloth-bound).

This handsome volume must find its way on to the shelves of all botanical institutes. It deals with algae, fungi, lichens, and bryophytes. It is not meant for the beginner, nor really for the specialist, but rather for the university teacher and general botanist who want the most recent information about the cellular structure and reproduction of these groups. Biology, or natural history, is eschewed, but there is an enormous amount on microscopic structure, particularly what used to be called cytological. This material is presented systematically, but the volume is not a systematic treatise. It lacks all pretence of taxonomic exposition with concise classification, though keys and tables to orders and families are given in various parts of the text and many new names are coined (without Latin diagnosis), as if there were not more than enough already! Likewise many new descriptive terms are coined, but there is neither glossary nor index, save to taxonomic groups. The table of contents is well-hidden in the last six pages of the massive volume. References are in foot-notes. Thus it is not an easy work to use.

The French school of cryptogamic botany tends to become more and more cytological. It is extremely useful, therefore, to have this displayed and summarized. At the same time it is annoying to find the cryptogams treated so academically with little regard to their lives. For this reason I do not think the treatise will survive as a great work; it may put many off. The author is too didactic and most of his profuse illustrations are too schematic. The first, on page 1, shows what is meant to be a typical plant-cell, but it is a dead one, plasmolysed and stained. No magnifications are given and the flagellum in all its intricacy outclasses *Macrocystis* in all its grandeur. The scheme of the basidiomycete life-cycle (Fig. 527) seems the height of nightmare, but it is what the author distils from his analysis of these organisms. On the same page, in Fig. 526, are shown various basidia but the characteristic shape and attachment of the basidiospore are omitted, and they cannot be distinguished from conidiophores.

The author is most at home with the algae (400 pages), next with the fungi, particularly the ascomycetes and lichens, and least with the bryophytes (67 pages) which, as Archegoniatae, seem a technical appendage to the volume rather than an integral part; the author, however, compares them with Rhodomelaceae. Since there is only passing reference to Church's work on algae, somatic organization is poorly dealt with. The emphasis is on the microscopic and reproductive. For the Brown Algae, in the widest sense, there are 100 pages on unicellular forms, and 34 on the multicellular; yet these are the greatest accomplishment of living cryptogams. Thus, the schematic picture of parenchymatous construction (Fig. 69, 7*b*) shows how little this achievement is understood. The presentation, however, is more up-to-date than Fritsch's and the illustrations are bolder, though in all cases rather coarse and without that brilliant delicacy of the traditional French algologists which Oltmanns was able to capture.

The same criticism can be levelled at the sections on fungi. Extremely useful are the detailed accounts of cell-structure and reproduction appertaining to many organisms which have not yet found their way into general mycology. While one is glad to have the author's intricate study of the ascus-apex systematically explained, one is annoyed to find so much of the work of others on the construction and functioning of fruit-bodies so abjectly neglected. That of Buller, Singer, or even myself, though cited in footnotes, need really not have been written for aught that appears in this treatise on cryptogams. 'Les Clavaires proprement dits' are quite outmoded and 'improprement dits'. Indeed, the whole section on 'higher basidiomycetes' is unsatisfactory and, in so learned a treatise, more is expected than the Friesian classification of agarics. The author promulgates 'Archeobasidiés' and 'Neobasidiés', without explaining the real nature of the basidium and without argument; future conclusions may well reverse the impression created by these names.

Why, then, do I recommend this book? First, it contains so much information that is scattered through the literature as well as much that is new. Secondly, it should spur others to reconsider nearly everything that it contains, unless this is to be the cryptogamic botany of the future.

E. J. H. CORNER

Micro-organisms as Allies. By C. L. Duddington. (London: Faber and Faber, 1961.) Pp. 256, 16 plates and 18 Text-figures. Price: 25*s.*

In this book Dr Duddington gives a comprehensive survey of the micro-organisms domesticated by man, from the yeasts, the use of which in baking and wine-making is as old as civilization, to the moulds, the powers of which to produce penicillin or cortisone are among the epics of modern scientific discovery. The introductory chapter provides an inventory of 'the livestock of industry'—yeasts, moulds and bacteria—and the rest are devoted to a fuller treatment of each organism. This includes clear and simple details of its structure and relevant physiology with an historical account of its usage and culture. The yeasts have pride of place. Traditional methods and materials for brewing, wine-making, distillation and bread-making are fully described, as well as new industrial methods for producing alcohol from wood. Modern methods of producing quantities of penicillin from vast cultures of mould, or lactic acid from cultures of bacteria, are added to the old crafts of cheese and vinegar making. The historical perspective illustrates once again the now familiar story of the incredible acceleration in technological achievements during the last 20 years, in sharp contrast to the patient secret maturing of centuries-old crafts and cultures.

In aiming at a wide audience Dr Duddington has made one or two concessions to popularity—morals drawn or a teleogical slant imposed, or more rarely a misleading over-simplification—which are perhaps unnecessary, especially as the story itself stands on merit. The printed diagrams are rather poor and do less than they might to clarify the text.

This is an informative and very readable book, which offers to the specialist in other fields as well as to the layman an initiation into the mysteries of that exciting clinical industrial world where fungi and bacteria are harnessed more and more for the relief of man's estate.

JEANNE MARTIN

The Organisation of Cells and other Organisms. By L. E. R. Picken. (Oxford University Press, 1960.) Pp. xxxvii + 629, 34 plates, 113 text-figures. Price: 84*s.*

In the opening words of his preface, Dr Picken says that his book may be regarded as a work of compilation addressed to postgraduate students and research workers; it covers a wide field and, with characteristic modesty, the author pays generous tribute to existing reviews of lesser scope; but, throughout its entire length, his book displays a level of scholarship and a clarity of exposition to which such articles seldom, if ever, attain.

As indicated by its title, the book is largely concerned with the structure of cells. It is essentially a treatise on molecular anatomy, and as such will be hailed with enthusiasm by biologists in the prime of life with time and energy to have made, or to make, themselves familiar with the principles of X-ray analysis, solid geometry and enzyme chemistry. Dr Picken is convinced that virology, bacteriology and immunology should now form the background knowledge of modern cytology; only then will it be possible to integrate the whole field of relevant material. Certainly without such a background it is impossible to appreciate the richness of the feast which Dr Picken's book provides. It may be that its full value will be even better appreciated in twenty years time than today. Each of the ten main chapters provides a masterly survey of the type of cell or organism concerned—and is written in a style which, to the reviewer at any rate, makes it unusually pleasant to read.

But, like the authors of many great books, Dr Picken will be fortunate if he escapes all criticism. Microbiology (née Cytology) is a popular subject and the number of papers published every year increases logarithmically. A review may be seriously out of date in 2 or 3 years time and some people may doubt how far a single author can hope to sift the wheat from the chaff over more than a very limited field. Such difficulties are inescapable and anyone reading this book in the hope that it will provide a pleasant table d'hôte introduction to modern cytology may be disappointed; each chapter has its own particular savour only to be appreciated by those with reasonably well-trained appetites. But, before reading any of the main chapters there is much to be said for reading—and indeed re-reading—the 'Introduction' and 'Envoi' where the author deals aesthetically and philosophically with what can otherwise become a rather sterile desert of factual knowledge.

It would be simple, but wrong, to subject this book to extravagant praise; it can, and will, stand by its own merits. The text covers five hundred relatively large pages and there are more than seventy pages of references. To complain about omissions would be unreasonable; nevertheless, one is left with a feeling that such a detailed and masterly description of structure tends to mask a picture of the cell as a dynamic system. In other fields of biology, structure and function have learnt to advance together with profit to all concerned. It may be that Dr Picken feels that the study of cell dynamics is best deferred until our knowledge of macro-molecular structure has reached a later stage. He does not entirely neglect the physiological approach, but seems more interested in statics than dynamics—more interested in the form of architecture of a complex factory than in the machinery which transforms raw material and energy into final products. Perhaps, one day, he will write a modern counterpart of Höber's *Physikalische Chemie der Zelle und Gewebe*.

The book is generously illustrated by half-tone plates many of which are, not unnaturally, reproductions of electron-micrographs; the majority are beyond criticism but others—together with some parts of the text—depend for their validity on the demonstration that the structures observed after fixation are a reliable guide to those present in the living state. The word 'artefact' appears on strangely few occasions.

As one might expect, the standard of typography and the large majority of the diagrams are excellent, but was it beyond the wit of man to put the full legends of the thirty-four half-tone plates elsewhere than at the beginning of the book? These legends are essential for an understanding of the plates and were it not for the intrinsic interest of the text, the inconvenience of constant back-referencing from text to plate and from plate to legend would be well nigh insufferable.

Dr Picken's objective has been to produce a synthetic picture of the organization of cells in terms of macro-molecular structure and texture. This he has achieved with conspicuous success. But he has done something more. He has shown that it is possible to present a great wealth of scientific knowledge and yet convey deep feeling for the intrinsic beauty of living things.

JAMES GRAY

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